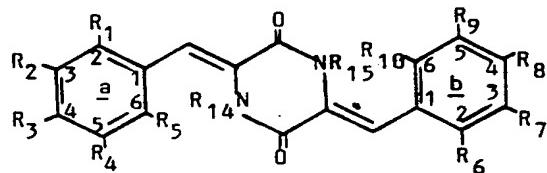


## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification 5 :</b> <b>C07D 241/08, A61K 31/495</b>		<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 94/04513</b> <b>(43) International Publication Date:</b> 3 March 1994 (03.03.94)
<b>(21) International Application Number:</b> PCT/GB93/01735			<b>(74) Agents:</b> WOODS, Geoffrey, Corlett et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).
<b>(22) International Filing Date:</b> 16 August 1993 (16.08.93)			
<b>(30) Priority data:</b> 9217331.9 14 August 1992 (14.08.92) GB			<b>(81) Designated States:</b> AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
<b>(71) Applicant (for all designated States except US):</b> XENOVA LIMITED [GB/GB]; 240 Bath Road, Slough, Berkshire SL1 4EF (GB).			
<b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> COLLINS, Mark, Anthony, David [GB/GB]; CHICARELLI-ROBINSON, Maria, Inês [BR/GB]; BRYANS, Justin, Stephen [GB/GB]; BROCCINI, Stephen, James [US/GB]; LATHAM, Christopher, John [GB/GB]; 240 Bath Road, Slough, Berkshire SL1 4EF (GB).			<b>Published</b> <i>With international search report.</i>

**(54) Title:** PHARMACEUTICALLY ACTIVE DIKETOPIPERAZINES

(A)

**(57) Abstract**

A diketopiperazine of formula (A) wherein each of R<sub>14</sub> and R<sub>15</sub>, which may be the same or different, is independently selected from hydrogen and C<sub>1</sub>-C<sub>6</sub> alkyl provided at least one of R<sub>14</sub> and R<sub>15</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl; and R<sub>1</sub> to R<sub>10</sub>, which may be the same or different, is independently selected from hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl unsubstituted or substituted by one or more hydrogen atoms, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> alkylthio, halogen, hydroxy, nitro, optionally substituted phenyl, cyano, -CH<sub>2</sub>OH, -CH<sub>2</sub>COOH, -CO<sub>2</sub>R<sup>11</sup>, -NHCOR<sup>11</sup>, -NHSO<sub>2</sub>R<sup>13</sup>, -SO<sub>2</sub>R<sup>13</sup>, -CON(R<sup>11</sup>)R<sup>12</sup>, -SOR<sup>13</sup>, -SO<sub>2</sub>N(R<sup>11</sup>R<sup>12</sup>), -N(R<sup>11</sup>R<sup>12</sup>), -O(CH<sub>2</sub>)<sub>n</sub>N(R<sup>11</sup>R<sup>12</sup>), -O(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>R<sup>11</sup>, -OCOR<sup>11</sup>, -CH<sub>2</sub>OCOR<sup>11</sup>, -CH<sub>2</sub>NHCOR<sup>11</sup>, -CH<sub>2</sub>NHCOOR<sup>13</sup>, -CH<sub>2</sub>SR<sup>11</sup>, -CH<sub>2</sub>SCOR<sup>11</sup>, -CH<sub>2</sub>S(O)<sub>m</sub>R<sup>13</sup> wherein m is 1 or 2, -CH<sub>2</sub>NHCO(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>R<sup>11</sup>, -N(R<sup>11</sup>)COR<sup>12</sup>, -NHCOCF<sub>3</sub>, -NHCO(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>R<sup>11</sup>, -NHCO(CH<sub>2</sub>)<sub>n</sub>OCOR<sup>11</sup> and -NHCO(CH<sub>2</sub>)<sub>n</sub>OR<sup>11</sup> wherein n is 0 or is an integer of from 1 to 6, each of R<sup>11</sup> and R<sup>12</sup> is independently H or C<sub>1</sub>-C<sub>6</sub> alkyl or, when R<sup>11</sup> and R<sup>12</sup> are attached to the same nitrogen atom, they may alternatively form with the nitrogen atom a saturated five or six-membered ring; and R<sup>13</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl; or any of R<sub>1</sub> and R<sub>2</sub>, R<sub>2</sub> and R<sub>3</sub>, R<sub>3</sub> and R<sub>4</sub> and R<sub>4</sub> and R<sub>5</sub>, or R<sub>6</sub> and R<sub>7</sub>, R<sub>7</sub> and R<sub>8</sub>, R<sub>8</sub> and R<sub>9</sub> and R<sub>9</sub> and R<sub>10</sub>, form together with the carbon atoms to which they are attached a benzene ring which is optionally substituted; and pharmaceutically acceptable salts and esters thereof; are modulators of multiple drug resistance.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

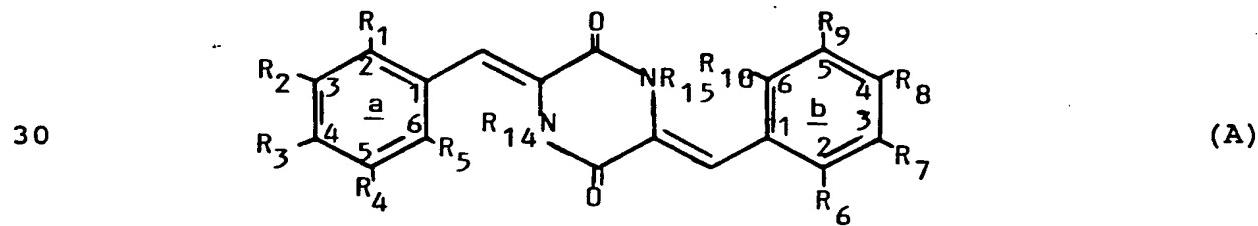
- 1 -

### Pharmaceutically active diketopiperazines

The present invention relates to compounds useful as modulators of multiple drug resistance (MDR), to their preparation and to pharmaceutical and veterinary compositions containing them.

The resistance of tumours to treatment with certain cytotoxic agents is an obstacle to the successful chemotherapeutic treatment of cancer patients. A tumour may acquire resistance to a cytotoxic agent used in a previous treatment. A tumour may also manifest intrinsic resistance, or cross-resistance, to a cytotoxic agent to which it has not previously been exposed, that agent being unrelated by structure or mechanism of action to any agent used in previous treatments of the tumour. These phenomena are referred to collectively as multiple drug resistance (MDR). Disadvantages of drugs which have so far been used to modulate MDR, termed resistance modifying agents or RMAs, are that they frequently possess a poor pharmacokinetic profile and/or are toxic at the concentrations required for MDR modulation.

25



35

wherein each of R<sub>14</sub> and R<sub>15</sub>, which may be the same or different, is independently selected from hydrogen and C<sub>1</sub>-

- 2 -

- $C_6$  alkyl provided at least one of  $R_{14}$  and  $R_{15}$  is  $C_1-C_6$  alkyl; and each of  $R_1$  to  $R_{10}$ , which may be the same or different, is independently selected from hydrogen,  $C_1-C_6$  alkyl unsubstituted or substituted by one or more halogen atoms,
- 5     $C_1-C_6$  alkoxy,  $C_1-C_6$  alkylthio, halogen, hydroxy, nitro, optionally substituted phenyl, -cyano,  $-CH_2OH$ ,  $-CH_2COOH$ ,  $-CO_2R^{11}$ ,  $-NHCOR^{11}$ ,  $-NHSO_2R^{13}$ ,  $-SO_2R^{13}$ ,  $-CON(R^{11}R^{12})$ ,  $-SOR^{13}$ ,  $-SO_2N(R^{11}R^{12})$ ,  $-N(R^{11}R^{12})$ ,  $-O(CH_2)_nN(R^{11}R^{12})$ ,  $-O(CH_2)_nCO_2R^{11}$ ,  $-OCOR^{11}$ ,  $-CH_2OCOR^{11}$ ,  $-CH_2NHCOR^{11}$ ,  $-CH_2NHCOOR^{13}$ ,  $-CH_2SR^{11}$ ,
- 10     $-CH_2SCOR^{11}$ ,  $-CH_2S(O)_mR^{13}$  wherein m is 1 or 2,  $-CH_2NHCO(CH_2)_nCO_2R^{11}$ ,  $-N(R^{11})COR^{12}$ ,  $-NHCOCF_3$ ,  $-NHCO(CH_2)_nCO_2R^{11}$ ,  $-NHCO(CH_2)_nOCOR^{11}$  and  $-NHCO(CH_2)_nOR^{11}$  wherein n is 0 or is an integer of from 1 to 6, each of  $R^{11}$  and  $R^{12}$  is independently H or  $C_1-C_6$  alkyl or, when  $R^{11}$  and  $R^{12}$  are attached to the
- 15    same nitrogen atom, they may alternatively form with the nitrogen atom a saturated five or six membered heterocyclic ring; and  $R^{13}$  is  $C_1-C_6$  alkyl; or any of  $R_1$  and  $R_2$ ,  $R_2$  and  $R_3$ ,  $R_3$  and  $R_4$  and  $R_4$  and  $R_5$ , or  $R_6$  and  $R_7$ ,  $R_7$  and  $R_8$ ,  $R_8$  and  $R_9$  and  $R_9$  and  $R_{10}$ , form together with the carbon atoms to which
- 20    they are attached a benzene ring which is optionally substituted; or a pharmaceutically acceptable salt or ester thereof; in the manufacture of a medicament for use as a modulator of multiple drug resistance.

The numerals 1 to 10 denote ring positions on the phenyl groups in formula A. The letters a and b refer to the two phenyl rings themselves.

When any two adjacent groups of  $R_1$  to  $R_{10}$  form, together with the carbon atoms to which they are attached, a benzene ring, that ring is either unsubstituted or it may 30 be substituted by any of the options specified above for  $R_1$  to  $R_{10}$ . The benzene ring forms, together with ring a or b respectively, an optionally substituted naphthalene ring structure.

When ring a or b is substituted phenyl, the benzene 35 ring may be substituted at any of the ortho, meta and para positions by one or more substituents, for example one, two or three substituents, which may be the same or different,

- 3 -

independently selected from the groups specified above for R<sub>1</sub> to R<sub>10</sub> other than hydrogen.

An alkyl group may be linear or branched, or may comprise a cycloalkyl group. A C<sub>1</sub>-C<sub>6</sub> alkyl group is typically a C<sub>1</sub>-C<sub>4</sub> alkyl group, for example a methyl, ethyl, propyl, i-propyl, n-butyl, sec-butyl, tert-butyl or cyclopropylmethyl group. A halogen is, for example, fluorine, chlorine, bromine or iodine. A C<sub>1</sub>-C<sub>6</sub> alkyl group substituted by halogen may be substituted by 1, 2 or 3 halogen atoms. It may be a perhaloalkyl group, for example trifluoromethyl.

A C<sub>1</sub>-C<sub>6</sub> alkoxy group is typically a C<sub>1</sub>-C<sub>4</sub> alkoxy group, for example a methoxy, ethoxy, propoxy, i-propoxy, n-butoxy, sec-butoxy or tert-butoxy group. A C<sub>1</sub>-C<sub>6</sub> alkylthio group is typically a C<sub>1</sub>-C<sub>4</sub> alkylthio group, for example methylthio, ethylthio, propylthio, i-propylthio, n-butylthio, sec-butylthio or tert-butylthio.

When R<sup>11</sup> and R<sup>12</sup> form a heterocyclic group together with the nitrogen atom to which they are attached, it is, for example, an N,N-tetramethylene group.

In compounds of formula A free rotation may occur at room temperature about the single bonds connecting rings a and b to the double bonds at positions 3 and 6 of the 2,5-piperazinedione ring. Positions 2 and 6, and positions 3 and 5, in both rings a and b can therefore be considered as equivalent. As a consequence the following pairs of substituents can be viewed as interchangeable: R<sub>1</sub> and R<sub>5</sub>; R<sub>2</sub> and R<sub>4</sub>; R<sub>6</sub> and R<sub>10</sub>; and R<sub>7</sub> and R<sub>9</sub>.

One of R<sub>14</sub> and R<sub>15</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl and the other is hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl. When R<sub>14</sub> and R<sub>15</sub> are both C<sub>1</sub>-C<sub>6</sub> alkyl they may be the same or different. Preferred C<sub>1</sub>-C<sub>6</sub> alkyl groups for R<sub>14</sub> and R<sub>15</sub> are Me, Et and cyclopropylmethyl. For example R<sub>14</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl and R<sub>15</sub> is H or C<sub>1</sub>-C<sub>6</sub> alkyl, or R<sub>15</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl and R<sub>14</sub> is H or C<sub>1</sub>-C<sub>6</sub> alkyl. In one embodiment R<sub>14</sub> is Me, Et or cyclopropylmethyl and R<sub>15</sub> is H, Me, Et or cyclopropylmethyl. In a second embodiment R<sub>15</sub> is Me, Et or

- 4 -

cyclopropylmethyl and R<sub>14</sub> is H, Me, Et or cyclopropylmethyl.

Preferably one of rings a and b is unsubstituted or is mono-substituted whilst the other ring is unsubstituted 5 or is substituted at one or more of positions 2 to 6. The ring which is mono-substituted may carry the substituent at any one of positions 2 to 6, for instance position 3 or 4, especially position 4. Thus for instance, when ring b is mono-substituted, one of R<sub>6</sub> to R<sub>10</sub> is other than hydrogen, 10 preferably R<sub>7</sub> or R<sub>8</sub>, especially R<sub>8</sub>. When ring a is mono- substituted, one of R<sub>1</sub> to R<sub>5</sub> is other than hydrogen, preferably R<sub>2</sub> or R<sub>3</sub>, especially R<sub>3</sub>. When one of rings a and b is mono-substituted the substituent R<sub>1</sub> to R<sub>5</sub>, or R<sub>6</sub> to R<sub>10</sub> respectively, is preferably selected from a halogen, for 15 instance fluorine; an alkoxy group, for instance OMe; and an acetamido group -NHAc in which Ac denotes acetyl.

When one of rings a and b is unsubstituted, or is mono-substituted as described in the above paragraph, the other ring may bear any desired substitution pattern. For 20 instance, the other ring may be unsubstituted or may be mono-, di- or tri-substituted at any of positions 2 to 6.

The said other ring may, for instance, be mono- substituted at any of positions 2 to 6. It may also be 2,3-, 2,4-, 2,5-, 2,6-, 3,4- or 3,5- disubstituted, or 25 2,3,4-, 2,3,5-, 2,3,6- or 3,4,5-trisubstituted. Thus, when the said other ring is a and is mono-substituted, four of R<sub>1</sub> to R<sub>5</sub> are hydrogen and one is other than hydrogen. When the said other ring is ring a and is disubstituted, three of R<sub>1</sub> to R<sub>5</sub> are hydrogen and two are other than hydrogen. 30 For example R<sub>1</sub> and R<sub>2</sub>, or R<sub>1</sub> and R<sub>3</sub>, or R<sub>1</sub> and R<sub>4</sub>, or R<sub>1</sub> and R<sub>5</sub>, or R<sub>2</sub> and R<sub>3</sub>, or R<sub>2</sub> and R<sub>4</sub> are other than hydrogen whilst, in each case, the other three of R<sub>1</sub> to R<sub>5</sub> are hydrogen.

When the said other ring is ring a and is 35 trisubstituted, two of R<sub>1</sub> to R<sub>5</sub> are hydrogen and three are other than hydrogen. For example, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub>, or R<sub>1</sub>, R<sub>2</sub> and R<sub>4</sub>, or R<sub>1</sub>, R<sub>2</sub> and R<sub>5</sub>, or R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are other than

- 5 -

hydrogen whilst, in each case, the other two of R<sub>1</sub> to R<sub>5</sub> are hydrogen.

When the said ring is b and is mono-substituted, four of R<sub>6</sub> to R<sub>10</sub> are hydrogen and one is other than hydrogen.

- 5 When the said other ring is b and is di-substituted, three of R<sub>6</sub> to R<sub>10</sub> are hydrogen and two are other than hydrogen. For example R<sub>6</sub> and R<sub>7</sub>, or R<sub>6</sub> and R<sub>8</sub>, or R<sub>6</sub> and R<sub>9</sub>, or R<sub>6</sub> and R<sub>10</sub>, or R<sub>7</sub> and R<sub>8</sub>, or R<sub>7</sub> and R<sub>9</sub>, are other than hydrogen whilst, in each case, the other three of R<sub>6</sub> to R<sub>10</sub> are 10 hydrogen. When the said other ring is b and is trisubstituted, two of R<sub>6</sub> to R<sub>10</sub> are hydrogen and three are other than hydrogen. For example R<sub>6</sub>, R<sub>7</sub> and R<sub>8</sub>, or R<sub>6</sub>, R<sub>7</sub> and R<sub>9</sub>, or R<sub>6</sub>, R<sub>7</sub> and R<sub>10</sub>, or R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> are other than 15 hydrogen whilst, in each case, the other two of R<sub>6</sub> to R<sub>10</sub> are hydrogen.

Alternatively, any two adjacent substituents in the said other ring may, together with the carbon atoms to which they are attached, complete a second benzene ring which is optionally substituted, thus forming an optionally 20 substituted naphthyl group with the said other ring. For instance, in ring a R<sub>1</sub> and R<sub>2</sub>, or R<sub>2</sub> and R<sub>3</sub> may form together with carbon atoms 2 and 3, or 3 and 4 respectively, an optionally substituted benzene ring which, in turn, forms with ring a a naphthyl group which is 25 unsubstituted or substituted by one or more groups specified above for R<sub>1</sub> to R<sub>10</sub>. In ring b R<sub>6</sub> and R<sub>7</sub>, or R<sub>7</sub> and R<sub>8</sub> may form, together with carbon atoms 2 and 3 or 3 and 4 respectively, an optionally substituted benzene ring which, in turn, forms with ring b a naphthyl group which is 30 unsubstituted or substituted by one or more groups specified above for R<sub>1</sub> to R<sub>10</sub>. Typically the naphthyl group in either case is unsubstituted or is monosubstituted at position 1,2,3 or 4 of the naphthalene ring structure, especially position 4. For example R<sub>1</sub> and R<sub>2</sub> together with 35 ring a, or R<sub>6</sub> and R<sub>7</sub> with ring b, form a 4-dimethylamino-1-naphthyl group.

In a preferred series of compounds of formula A each

- 6 -

of R<sub>6</sub> to R<sub>10</sub> is hydrogen. In another preferred series of compounds, one of R<sub>6</sub> to R<sub>10</sub> is selected from hydroxy, alkoxy, NHCOR<sup>11</sup>, -CO<sub>2</sub>R<sup>11</sup>, -N(R<sup>11</sup>R<sup>12</sup>), -O(CH<sub>2</sub>)<sub>n</sub>N(R<sup>11</sup>R<sup>12</sup>), -SO<sub>2</sub>R<sup>13</sup>, -CON(R<sup>11</sup>R<sup>12</sup>), NO<sub>2</sub>, -SO<sub>2</sub>N(R<sup>11</sup>R<sup>12</sup>), -SOR<sup>13</sup>, -N(R<sup>11</sup>)COR<sup>12</sup> and

5 halogen and the other four of R<sub>6</sub> to R<sub>10</sub> are H. Alkoxy may be, for instance, OMe or OBu<sup>n</sup>. NHCOR<sup>11</sup> is typically -NHAc. CO<sub>2</sub>R<sup>11</sup> is typically -COOH or -COOMe. N(R<sup>11</sup>R<sup>12</sup>) is typically NMe<sub>2</sub> or N,N-tetramethylene. CON(R<sup>11</sup>R<sup>12</sup>) may be -CONH<sub>2</sub>. SO<sub>2</sub>R<sup>13</sup> is typically SO<sub>2</sub>Me, SO<sub>2</sub>N(R<sup>11</sup>R<sup>12</sup>) is for example

10 -SO<sub>2</sub>NMe<sub>2</sub>. SOR<sup>13</sup> may be SOMe and -N(R<sup>11</sup>)COR<sup>12</sup> may be -NMeCOBu<sup>t</sup>. Halogen is typically F or Cl. Preferably R<sub>8</sub> is alkoxy, especially OMe or OBu<sup>n</sup>; NHCOR<sup>11</sup>, especially -NHAc; -CO<sub>2</sub>R<sup>11</sup>, especially -CO<sub>2</sub>H or

-CO<sub>2</sub>Me; -CON(R<sup>11</sup>R<sup>12</sup>) especially -CONH<sub>2</sub>; NO<sub>2</sub>; N(R<sup>11</sup>R<sup>12</sup>)

15 especially NMe<sub>2</sub> or N,N-tetramethylene; -SOR<sup>13</sup> especially -SOMe; -SO<sub>2</sub>N(R<sup>11</sup>R<sup>12</sup>) especially -SO<sub>2</sub>NMe<sub>2</sub> or halogen, especially F or Cl; and each of R<sub>6</sub>, R<sub>7</sub>, R<sub>9</sub> and R<sub>10</sub> is H.

In the above-mentioned series of preferred compounds R<sub>1</sub> to R<sub>5</sub> are all hydrogen, or one or two of R<sub>1</sub> to R<sub>5</sub> are other than hydrogen whilst the others are hydrogen. For instance one of R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> is other than hydrogen. Alternatively R<sub>1</sub> and R<sub>3</sub>, or R<sub>2</sub> and R<sub>3</sub>, are other than hydrogen. Preferred values for the one or two of R<sub>1</sub> to R<sub>5</sub> which is or are other than hydrogen include alkoxy such as

25 OMe or OBu<sup>n</sup>, halogen such as Cl or F, hydroxy, -N(R<sup>11</sup>R<sup>12</sup>), -CO<sub>2</sub>R<sup>11</sup>, -CH<sub>2</sub>SCOR<sup>13</sup>, -CH<sub>2</sub>SR<sup>11</sup>, -NHCOR<sup>11</sup>, -O(CH<sub>2</sub>)<sub>n</sub>N(R<sup>11</sup>R<sup>12</sup>), -O(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>R<sup>11</sup>, -CH<sub>2</sub>NHCO(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>R<sup>11</sup>, -NHCOCH<sub>2</sub>OR<sup>11</sup>, -NHCOCH<sub>2</sub>OCOR<sup>13</sup>, -CH<sub>2</sub>NHCOOR<sup>13</sup> and CF<sub>3</sub>. It is also preferred for R<sub>1</sub> and R<sub>2</sub>, R<sub>2</sub> and R<sub>3</sub>, R<sub>3</sub> and R<sub>4</sub> or R<sub>4</sub> and R<sub>5</sub> to form,

30 together with the carbon atoms to which they are attached, a benzene ring.

Particularly preferred compounds are those wherein R<sub>6</sub>, R<sub>7</sub>, R<sub>9</sub> and R<sub>10</sub> are each H, R<sub>8</sub> is selected from H, OMe -NHAc, -CO<sub>2</sub>H, -CO<sub>2</sub>Me, -COHN<sub>2</sub>, NO<sub>2</sub>, -NMe<sub>2</sub>, N,N-tetramethylene,

35 SO<sub>2</sub>Me, -SOMe and -SO<sub>2</sub>NMe<sub>2</sub> and each of R<sub>1</sub> to R<sub>5</sub> is as specified above. In these preferred compounds R<sup>1</sup> to R<sup>5</sup> are preferably each independently selected from H, halogen,

- 7 -

- hydroxy,  $C_1-C_6$  alkoxy, nitro,  $-\text{CH}_2\text{SCOR}^{13}$ ,  $-\text{CH}_2\text{SR}^{11}$ ,  $-\text{CO}_2\text{R}^{11}$ ,  
 $-\text{OCOR}^{13}$ ,  $\text{CF}_3$ ,  $-\text{O}(\text{CH}_2)_n\text{N}(\text{R}^{11}\text{R}^{12})$ ,  $-\text{O}(\text{CH}_2)_n\text{CO}_2\text{R}^{11}$ ,  
 $-\text{CH}_2\text{NHCO}(\text{CH}_2)_n\text{CO}_2\text{R}^{11}$ ,  $-\text{NHCO}(\text{CH}_2)_n\text{OR}^{11}$ ,  $-\text{N}(\text{R}^{11}\text{R}^{12})$ ,  
 $-\text{NHCO}(\text{CH}_2)_n\text{OCOR}^{11}$ ,  $-\text{NHCO}(\text{CH}_2)_n\text{CO}_2\text{R}^{11}$  and  $-\text{CH}_2\text{NHCO}_2\text{R}^{13}$  or  $R_1$  and  
5  $R_2$ ,  $R_2$  and  $R_3$ ,  $R_3$  and  $R_4$ , or  $R_4$  and  $R_5$ , form with the carbon atoms to which they are attached an optionally substituted benzene ring. Still more preferably,  $R_1$  and  $R_2$  are independently H, nitro or halogen,  $R_3$  is H, hydroxy,  
 $-\text{O}(\text{CH}_2)_n\text{N}(\text{R}^{11}\text{R}^{12})$ ,  $-\text{OCOR}^{13}$ ,  $-\text{O}(\text{CH}_2)_n\text{CO}_2\text{R}^{11}$ ,  $-\text{CH}_2\text{NHCO}(\text{CH}_2)_n\text{CO}_2\text{R}^{11}$ ,  
10  $C_1-C_6$  alkoxy,  $-\text{NHCO}(\text{CH}_2)_n\text{OR}^{11}$ ,  $-\text{NHCO}(\text{CH}_2)\text{OCOR}^{11}$ ,  $-\text{N}(\text{R}^{11}\text{R}^{12})$ ,  
 $-\text{CH}_2\text{NHCO}_2\text{R}^{13}$ ,  $-\text{CH}_2\text{SR}^{11}$  or  $-\text{NHCOOR}^{11}$ ;  $R_4$  is H, halogen,  $C_1-C_6$  alkoxy,  $-\text{CH}_2\text{SCOR}^{13}$ ,  $-\text{CH}_2\text{SR}^{11}$  or  $-\text{CO}_2\text{R}^{11}$ ; and  $R_5$  is H, nitro or halogen; or  $R_2$  and  $R_3$ ,  $R_3$  and  $R_4$  or  $R_4$  and  $R_5$  form, together with the carbon atoms to which they are attached, an  
15 optionally substituted benzene ring.

In one embodiment  $R^8$  is NHAc, each of  $R_6$ ,  $R_7$ ,  $R_9$  and  $R_{10}$  is H;  $R_1$  is H or halogen such as Cl or F;  $R_2$  is H,  $R_3$  is halogen such as F or Cl,  $C_1-C_6$  alkoxy such as OMe,  $-\text{N}(\text{R}^{11}\text{R}^{12})$  such as NMe<sub>2</sub> or  $-\text{NHCOOR}^{13}$  such as  $-\text{NHCOOBu}^t$ ;  $R_4$  is H and  $R_5$  is halogen such as F, Cl, Br, or is CF<sub>3</sub>.

In a second embodiment  $R^8$  is OMe, each of  $R_6$ ,  $R_7$ ,  $R_9$  and  $R_{10}$  is H;  $R^1$  is H, nitro or halogen such as Cl;  $R^2$  is H;  $R^3$  is H, hydroxy,  $-\text{OCOR}^{13}$  such as OAc,  $-\text{NHCO}(\text{CH}_2)_n\text{OCOR}^{11}$  such as  $-\text{NHCOCH}_2\text{OAc}$  or  $-\text{NHCOCH}_2\text{OR}^{11}$  such as  $-\text{NHCOCH}_2\text{OH}$ ;  $R_4$  is H and  $R_5$  is H or halogen such as F or Cl; or  $R_2$  and  $R_3$  form a benzene ring together with the carbon atoms to which they are attached.

In a third embodiment each of  $R_1$ ,  $R_6$ ,  $R_7$ ,  $R_8$ ,  $R_9$  and  $R_{10}$  is H;  $R_2$  is H,  $-\text{CH}_2\text{SCOR}^{13}$  such as  $-\text{CH}_2\text{SAC}$  or  $-\text{CH}_2\text{SR}^{11}$  such as

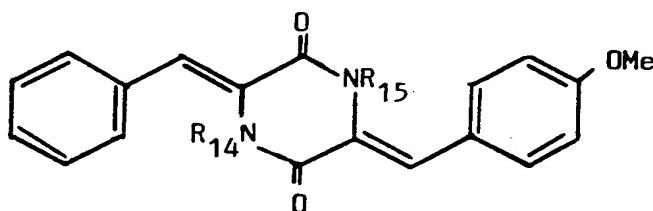
$-\text{CH}_2\text{SH}$ ;  $R_3$  is  $-\text{CH}_2\text{SR}^{11}$  such as  $-\text{CH}_2\text{SMe}$ ,  $-\text{CH}_2\text{SCOR}^{13}$  such as  $-\text{CH}_2\text{SAC}$ ,  $-\text{NHCO}(\text{CH}_2)_n\text{CO}_2\text{R}^{11}$  such as  $-\text{NHCO}(\text{CH}_2)_3\text{CO}_2\text{Me}$ ,  $-\text{O}(\text{CH}_2)_n\text{CO}_2\text{R}^{11}$  such as  $-\text{O}(\text{CH}_2)_4\text{CO}_2\text{H}$ ,  $-\text{O}(\text{CH}_2)_n\text{N}(\text{R}^{11}\text{R}^{12})$  such as  $-\text{O}(\text{CH}_2)_3\text{NMe}_2$ , or  $-\text{N}(\text{R}^{11}\text{R}^{12})$  such as NMe<sub>2</sub>; and  $R_4$  and  $R_5$  are both H or both form, together with the carbon atoms to which they are attached, a benzene ring.

In one embodiment of the invention the compound of

- 8 -

formula A has the following formula (Aa):

5



(Aa)

wherein each of R<sub>14</sub> and R<sub>15</sub>, which may be the same or different, is independently H or CH<sub>3</sub> provided at least one 10 is CH<sub>3</sub>.

Certain diketopiperazines have been disclosed as having utility as bioactive agents. Yokoi *et al* in J. Antibiotics vol XLI No. 4, pp 494-501 (1988) describe structure-cytotoxicity relationship studies on a series of 15 diketopiperazines related to neihumycin, a compound obtained from the micro-organism Micromonospora neihuensis. Kamei *et al* in J. Antibiotics vol XLIII No. 8, 1018-1020 disclose that two diketopiperazines, designated piperafizines A and B, have utility as potentiators of the 20 cytotoxicity of vincristine.

General formula A embraces diketopiperazines which are novel. Accordingly, the present invention provides a diketopiperazine of formula (A) as defined above, or a pharmaceutically acceptable salt or ester thereof; with the 25 exception of compounds wherein:

- (i) each of R<sub>1</sub> to R<sub>10</sub> is H; and
- (ii) R<sub>14</sub> and R<sub>15</sub> are both Me, R<sub>8</sub> is OMe and the rest of R<sub>1</sub> to R<sub>10</sub> are H.

Examples of specific compounds of the invention are 30 as follows. The compound numbering is adhered to in the rest of the specification:

(3Z,6Z)-3-benzylidene-6-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione (compound 1);

(3Z,6Z)-6-benzylidene-3-(4-methoxybenzylidene)-1-methyl-

35 2,5-piperazinedione (compound 121);

(3Z,6Z)-3,6-dibenzylidene-1-methyl-2,5-piperazinedione (compound 122);

- 9 -

- 4-((3Z,6Z)-3-benzylidene-1-methyl-2,5-dioxopiperazin-6-ylidene)methylbenzoic acid, methyl ester (compound 124);  
4-((3Z,6Z)-3-benzylidene-1-methyl-2,5-dioxopiperazin-6-ylidene)methylbenzoic acid (compound 125);  
5 (3Z,6Z)-3-(3-hydroxymethylbenzylidene)-6-benzylidene-1,4-dimethyl-2,5-piperazinedione (compound 126);  
(3Z,6Z)-3-benzylidene-1-methyl-6-(4-nitrobenzylidene)-2,5-piperazinedione (compound 127);  
N,N-tetramethylene-4-((3Z,6Z)-3-benzylidene-1-methyl-2,5-dioxopiperazin-6-ylidene)methylbenzamide (compound 128);  
10 (3Z,6Z)-6-(4-aminobenzylidene)-3-benzylidene-1-methyl-2,5-piperazinedione (compound 129);  
((3Z,6Z)-3-Benzylidene-1-methyl-2,5-dioxopiperazin-6-ylidene)methylbenzamide (compound 130);  
15 (3Z,6Z)-3-(4-Acetamidobenzylidene)-6-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione (compound 131);  
(3Z,6Z)-3-(2,6-Dichlorobenzylidene)-6-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione (compound  
20 132);  
(3Z,6Z)-6-(4-Methoxybenzylidene)-1-methyl-3-(2-nitrobenzylidene)-2,5-piperazinedione (compound 133);  
(3Z,6Z)-6-(4-Methoxybenzylidene)-1-methyl-3-(4-N-methylacetamidobenzylidene)-2,5-piperazinedione (compound  
25 134);  
(3Z,6Z)-6-(2,6-Dichlorobenzylidene)-3-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione (compound 135);  
(3Z,6Z)-3-(4-Methoxybenzylidene)-1-methyl-6-(2-nitrobenzylidene)-2,5-piperazinedione (compound 136);  
30 (3Z,6Z)-6-(4-Acetamidobenzylidene)-3-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione (compound 137);  
(3Z,6Z)-3-(4-(3-N,N-Dimethylaminopropoxy)benzylidene)-6-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione,  
35 hydrochloride (compound 138)  
(3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(4-

- 10 -

- trifluoromethylbenzylidene)-2,5-piperazinedione (compound 139)
- (3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(1-naphthylmethylene)-2,5-piperazinedione (compound 140)
- 5 (3Z,6Z)-6-Benzylidene-3-(4-dimethylaminobenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 141);  
(3Z,6Z)-6-Benzylidene-3-(2-methoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 142);  
(3Z,6Z)-3-(4-Aminobenzylidene)-6-benzylidene-1,4-dimethyl-2,5-piperazinedione (compound 143);  
10 (3Z,6Z)-6-Benzylidene-3-(2-fluorobenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 144)  
(3Z,6Z)-6-Benzylidene-3-(4-fluorobenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 145)
- 15 (3Z,6Z)-6-Benzylidene-3-(2,4-difluorobenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 146)  
(3Z,6Z)-3-(4-Acetoxyethylbenzylidene)-6-benzylidene-1,4-dimethyl-2,5-piperazinedione (compound 147)  
(3Z,6Z)-3-(3-Acetoxyethylbenzylidene)-6-benzylidene-1,4-dimethyl-2,5-piperazinedione (compound 148)
- 20 (3Z,6Z)-6-Benzylidene-3-(4-ethoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 149)  
(3Z,6Z)-3,6-Dibenzylidene-1,4-dimethyl-2,5-piperazinedione (compound 150)
- 25 (3Z,6Z)-6-Benzylidene-3-(2,6-dichlorobenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 151);  
(3Z,6Z)-6-(4-aminobenzylidene)-3-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione (compound 152);  
(3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(4-N-
- 30 methylacetamidobenzylidene)-2,5-piperazinedione (compound 153)  
(3Z,6Z)-6-Benzylidene-3-(3,4-dichlorobenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 154)  
(3Z,6Z)-6-Benzylidene-3-(3-chlorobenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 155)
- 35 (3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(4-methylsulfinylbenzylidene)-2,5-piperazinedione (compound

- 11 -

156)

N,N-Dimethyl-4-((3Z,6Z)-6-Benzylidene-1,4-dimethyl-2,5-dioxopiperazin-3-ylidene)methylbenzenesulfonamide (compound 157)

5 (3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(2-N-methyltrimethylacetamido benzylidene)-2,5-piperazinedione (compound 158)

(3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(4-phenylbenzylidene)-2,5-piperazinedione (compound 159)

10 4-((3Z,6Z)-6-Benzylidene-1,4-dimethyl-2,5-dioxopiperazin-3-ylidene)methylbenzoic acid, methyl ester (compound 160)  
(3Z,6Z)-6-Benzylidene-3-(4-bromobenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 161)

(3Z,6Z)-3-(2,4-Difluorobenzylidene)-6-(4-

15 methoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 162)

(3Z,6Z)-3-(4-Bromobenzylidene)-6-(4-methoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione (compound 163)

(3Z,6Z)-3-(4-Fluorobenzylidene)-6-(4-methoxybenzylidene)-

20 1,4-dimethyl-2,5-piperazinedione (compound 164)

(3Z,6Z)-3-(2,6-Dichlorobenzylidene)-1,4-dimethyl-6-(2-nitrobenzylidene)-2,5-piperazinedione (compound 165)

(3Z,6Z)-6-Benzylidene-1-cyclopropylmethyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (compound 166)

25 (3Z,6Z)-3-Benzylidene-1-cyclopropylmethyl-6-(4-methoxybenzylidene)-2,5-piperazinedione (compound 167)

(3Z,6Z)-6-Benzylidene-1-cyclopropylmethyl-3-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione (compound 168)

30 (3Z,6Z)-3,6-Dibenzylidene-1-ethyl-4-methyl-2,5-piperazinedione (compound 169)

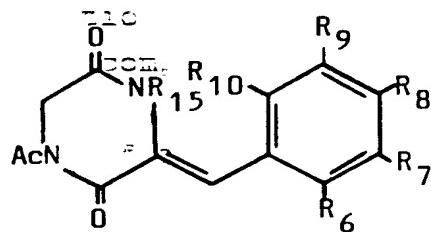
(3Z,6Z)-3-Benzylidene-1-cyclopropylmethyl-6-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione (compound 170)

35 Compounds of formula A, both known and novel, may be prepared by a process which comprises either (i) condensing compound of formula (I)

- 12 -

prepared by a process which comprises either (i) condensing compound of formula (I)

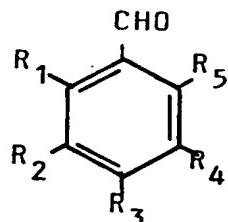
5



(I)

10 wherein R<sub>6</sub> to R<sub>10</sub> and R<sub>15</sub> are as defined above and are optionally protected, with a compound of formula (II):

15



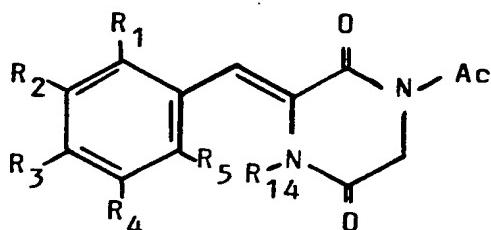
(II)

20

wherein R<sub>1</sub> to R<sub>5</sub> are defined above and are optionally protected, in the presence of a base in an organic solvent, thereby obtaining a compound of formula A in which R<sub>14</sub> is hydrogen; or (ii) condensing a compound of formula (I'):

25

30



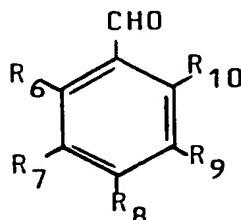
(I')

35 wherein R<sub>1</sub> to R<sub>5</sub> and R<sub>14</sub> are as defined above and are

- 13 -

optionally protected, with a compound of formula (III):

5



(III)

10

wherein R<sub>6</sub> to R<sub>10</sub> are as defined above and are optionally protected, in the presence of a base in an organic solvent, thereby obtaining a compound of formula A in which R<sub>15</sub> is hydrogen; and, in either case (i) or (ii), if desired, 15 converting the resulting compound of formula A in which R<sub>14</sub> or R<sub>15</sub>, respectively, is hydrogen into a corresponding compound of formula A in which R<sub>14</sub> and R<sub>15</sub>, respectively, is a C<sub>1</sub>-C<sub>6</sub> alkyl group, by treatment with an alkylating agent; and/or if required, removing optionally present protecting 20 groups and/or, if desired, converting one compound of formula A into another compound of formula A, and/or, if desired, converting a compound of formula A into a pharmaceutically acceptable salt or ester thereof, and/or, if desired, converting a salt or ester into a free 25 compound, and/or, if desired, separating a mixture of isomers of compounds of formula A into the single isomers.

A compound of formula A produced directly by the condensation reaction between (I) and (II) or (I') and (III) may be modified, if desired, by converting one or 30 more of groups R<sub>1</sub> to R<sub>10</sub> into different groups R<sub>1</sub> to R<sub>10</sub>. These optional conversions may be carried out by methods known in themselves. For example, a compound of formula A in which one or more of R<sub>1</sub> to R<sub>10</sub> is an ester group may be converted to a compound of formula A wherein the 35 corresponding substituent is a free -COOH group, by acid or alkaline hydrolysis at a suitable temperature, for example from ambient temperature to 100°C.

- 14 -

A compound of formula A in which one or more of R<sub>1</sub> to R<sub>10</sub> is a -CO<sub>2</sub>H group may be converted into a compound of formula A wherein the corresponding substituent is esterified by esterification, for example by treating the 5 carboxylic acid with a suitable C<sub>1</sub>-C<sub>6</sub> alkyl alcohol in the presence of 1,3-dicyclohexylcarbodiimide in an inert solvent.

A compound of formula A in which one or more of R<sub>1</sub> to R<sub>10</sub> is a free -CO<sub>2</sub>H group may be converted into a compound 10 of formula A in which the corresponding substituent is a group -CON(R<sup>11</sup>R<sup>12</sup>), wherein R<sup>11</sup> and R<sup>12</sup> are as defined above, for example by treatment with ammonia or an amine in the presence of 1,3-dicyclohexylcarbodiimide in an inert solvent.

15 A compound of formula A in which one or more of R<sub>1</sub> to R<sub>10</sub> is a free -CO<sub>2</sub>H group may be converted into a compound of formula A wherein the corresponding substituent is a -CH<sub>2</sub>OH group by reduction, for example using borane in a suitable solvent such as tetrahydrofuran.

20 A compound of formula A in which one or more of R<sub>1</sub> to R<sub>10</sub> is a nitro group may be converted into a compound of formula A in which the corresponding substituent is an amino group by reduction under standard conditions, for example by catalytic hydrogenation.

25 Protecting groups for R<sub>1</sub> to R<sub>10</sub> in any of the compounds of formulae (I), (I'), (II) and (III) are optionally introduced prior to step (i) or step (ii) when any of groups R<sub>1</sub> to R<sub>10</sub> are groups which are sensitive to the condensation reaction conditions or incompatible with 30 the condensation reaction, for example a -COOH, -CH<sub>2</sub>OH or amino group. The protecting groups are then removed at the end of the process. Any conventional protecting group suitable for the group R<sub>1</sub> to R<sub>10</sub> in question may be employed, and may be introduced and subsequently removed by 35 well-known standard methods.

The condensation reaction between compounds (I) and (II) or (I') and (III) is suitably performed in the

- 15 -

presence of a base which is potassium t-butoxide, sodium hydride, potassium carbonate, sodium carbonate, caesium carbonate, sodium acetate, potassium fluoride on alumina, or triethylamine in a solvent such as dimethylformamide, or  
5 in the presence of potassium t-butoxide in t-butanol or a mixture of t-butanol and dimethylformamide. The reaction is typically performed at a temperature from 0°C to the reflux temperature of the solvent.

The alkylation of a compound of formula A wherein R<sub>14</sub> or R<sub>15</sub> is H is carried out using an appropriate conventional alkylating agent such as a haloalkane, for example an iodoalkane, or a dialkylsulphate, in the presence of a base in an organic solvent. The base may be, for example, sodium hydride, sodium carbonate or potassium carbonate. A suitable solvent is then DMF. Another suitable base is aqueous sodium hydroxide, in which case a suitable cosolvent is, for example, dioxan, THF or DMF.  
10  
15

The compounds of formula (I) may be prepared by a process comprising reacting 1,4-diacetyl-2,5-piperazinedione with a compound of formula (III) as defined above, in the presence of a base in an organic solvent, thereby obtaining a compound of formula (I) wherein R<sub>15</sub> is hydrogen; and, if desired, treating the resulting compound of formula (I) with an alkylating agent to obtain a compound of formula (I) in which R<sub>15</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group. Similarly, the compounds of formula (I') may be prepared by a process which comprises reacting 1,4-diacetyl-2,5-piperazinedione with a compound of formula (II) as defined above, in the presence of a base in an organic solvent, thereby obtaining a compound of formula (I') in which R<sub>14</sub> is hydrogen; and, if desired, treating the resulting compound of formula (I') with an alkylating agent to obtain a compound of formula (I') in which R<sub>14</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group.  
20  
25  
30

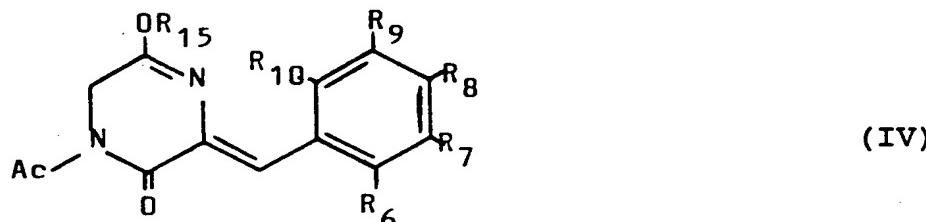
If necessary, the resulting compound of formula (I) or (I') can be separated from other reaction products by chromatography.  
35

- 16 -

The reaction of 1,4-diacetyl-2,5-piperazinedione with the compound of formula (III) or (II) is suitably performed under the same conditions as described above for the condensation between compounds (I) and (II), or (I') and 5 (III).

The alkylation of a compound of formula (I) in which R<sub>15</sub> is hydrogen, or a compound of formula (I') in which R<sub>14</sub> is hydrogen, is suitably carried out using the same conventional alkylating agents and under the same 10 conditions as described above for the alkylation of compounds of formula (A) in which R<sub>14</sub> is hydrogen. The alkylation step in the case of a compound (I) where R<sub>15</sub> is hydrogen typically gives rise to a mixture of the compound of formula (I) in which R<sub>15</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group and its 15 isomer of the following formula (IV) in which R<sub>15</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group:

20

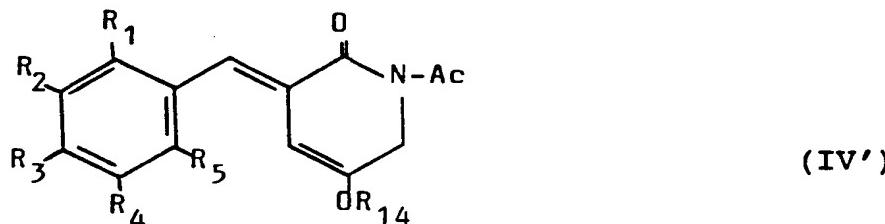


25

The alkylation step in the case of a compound (I') where R<sub>14</sub> is hydrogen typically gives rise to a mixture of the compound of formula (I') where R<sub>14</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group and its isomer of formula (IV') where R<sub>14</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group:

30

35



- 17 -

The mixture of compounds (I) and (IV), where R<sub>15</sub> is other than hydrogen, or compounds (I') and (IV'), where R<sub>14</sub> is other than hydrogen, can readily be separated by chromatography, for example on silica gel. Suitable 5 eluants include ethyl acetate and hexane, or methanol and dichloromethane.

The substituted benzaldehydes of formulae (II) and (III) are known compounds or can be prepared from readily available starting materials by conventional methods. The 10 1,4-diacetyl-2,5-piperazinedione used as a starting material in the preparation of compounds of formula (I) may be prepared by treating 2,5-piperazinedione (glycine anhydride) with an acetylating agent. The acetylation may be performed using any conventional acetylating agent, for 15 example acetic anhydride under reflux or, alternatively, acetic anhydride at a temperature below reflux in the presence of 4-dimethylaminopyridine.

Compounds of formula (I) wherein R<sub>15</sub> is H may also be prepared by the microwave irradiation of a mixture 20 comprising 1,4-diacetyl-2,5-piperazinedione, a compound of formula (III) and potassium fluoride on alumina (as base) in the absence of solvent.

Compounds of formula (I) wherein R<sub>15</sub> is H may alternatively be prepared directly from 2,5-piperazinedione 25 (glycine anhydride) by a process which comprises treating the 2,5-piperazinedione with a mixture comprising a compound of formula (III), sodium acetate and acetic anhydride at an elevated temperature, for example under reflux.

30 Compounds of formula (I') wherein R<sub>14</sub> is H may be prepared by analogous processes, replacing compound (III) in each case by a compound of formula (II).

Compounds of formula A may also be prepared by a process comprising the microwave irradiation of (i) a 35 mixture comprising a compound of formula (I) as defined above wherein R<sub>15</sub> is H or C<sub>1</sub>-C<sub>6</sub> alkyl, a compound of formula (II) and potassium fluoride on alumina, or (ii) a mixture

- 18 -

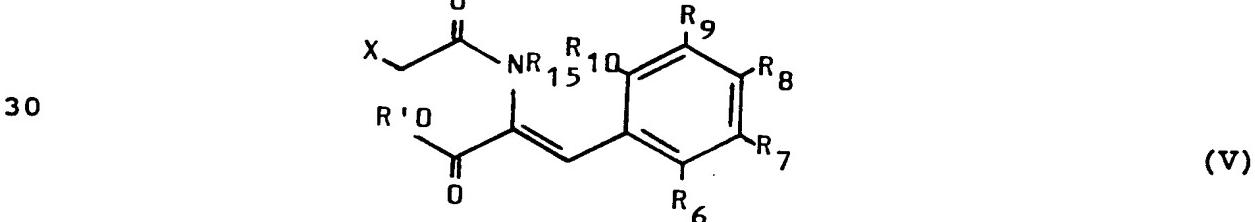
- comprising a compound of formula (I') wherein R<sub>14</sub> is H or C<sub>1</sub>-C<sub>6</sub> alkyl a compound of formula (III) and potassium fluoride on alumina, or (iii) a mixture comprising 1,4-diacetyl piperazine-2,5-dione, a compound of formula (II), a
- 5 compound of formula (III) and potassium fluoride on alumina. The irradiation is performed in the absence of a solvent. The resulting compound in which R<sub>14</sub> and R<sub>15</sub> are both H may then be alkylated using an appropriate alkylating agent, for example as described above.
- 10 Compounds of formula A may also be obtained directly by a process which comprises condensing together 1,4-diacetyl-2,5-piperazinedione, a compound of formula (II) and a compound of formula (III) in the presence of a base in an organic solvent. Suitable bases, solvents and
- 15 reaction conditions are as described above for the condensation reaction between, for example, compounds (I) and (II).

An alternative direct process for the preparation of compounds of formula A comprises condensing together 2,5-

20 piperazinedione, a compound of formula (II) and a compound of formula (III) in the presence of sodium acetate and acetic anhydride at elevated temperature, for example under reflux.

An alternative process for the preparation of

25 compounds of formula (I) comprises treating a compound of formula (V):



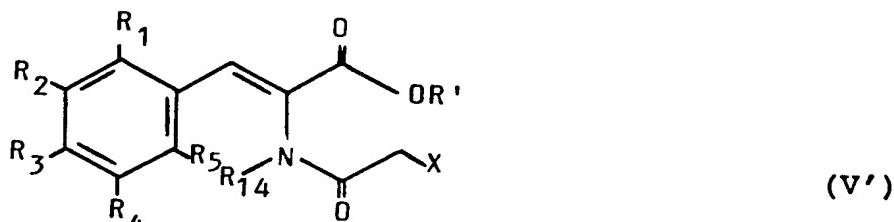
- 35 wherein R<sub>6</sub> to R<sub>10</sub> are as defined above, X is a halogen and R' is a C<sub>1</sub>-C<sub>6</sub> alkyl group, with ammonia followed by acetic anhydride.

- 19 -

Compounds of formula (I') may be prepared by an analogous process which comprises treating a compound of formula (V'):

5

10



wherein R<sub>1</sub> to R<sub>5</sub>, X and R' are as defined above, with ammonia followed by acetic anhydride.

15 X in formula (V) or (V') is typically iodine. R' is, for example, a C<sub>1</sub>-C<sub>4</sub> alkyl group such as a methyl, ethyl, propyl, i-propyl, butyl, sec-butyl or tert-butyl group.

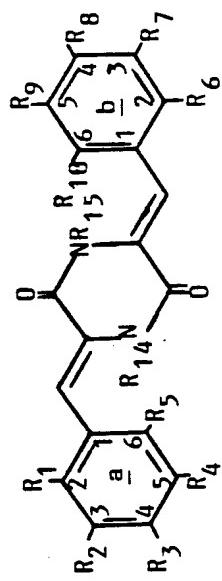
A review of synthetic approaches to unsaturated 3-monosubstituted and 3,6-disubstituted-2,5-piperazinediones 20 is provided in Heterocycles, 1983, 20, 1407 (C.Shin).

Compounds of formula (A) may be converted into pharmaceutically acceptable salts, and salts may be converted into the free compound, by conventional methods. Suitable salts include salts with pharmaceutically 25 acceptable, inorganic or organic, bases. Examples of inorganic bases include ammonia and carbonates, hydroxides and hydrogen carbonates of group I and group II metals such as sodium, potassium, magnesium and calcium. Examples of organic bases include aliphatic and aromatic amines such as 30 methylamine, triethylamine, benzylamine, dibenzylamine or α-or β-phenylethylamine, and heterocyclic bases such as piperidine, 1-methylpiperidine and morpholine.

Compounds of formula (A) may also be converted into pharmaceutically acceptable esters. Suitable esters 35 include branched or unbranched, saturated or unsaturated C<sub>1</sub>-C<sub>6</sub> alkyl esters, for example methyl, ethyl and vinyl esters.

- 20 -

Preferred compounds of formula A are depicted by means of their substitution patterns and identified by compound number in Table 1 which follows. Characterising data for the compounds are set out in Table 5 in Example 5 16. Diketopiperazine derivatives which are N-unsubstituted in the piperazine ring and can be converted by alkylation into compounds of formula A are depicted by substitution pattern in Table 2.

TABLE 1

COMPOUND NO.	R <sub>14</sub>	R <sub>15</sub>	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>
121	Me	H	H	H	H	H	H	H	H	OMe	H	H
122	Me	H	H	H	H	H	H	H	H	H	H	H
123	H	Me	H	H	H	H	H	H	H	OH	H	H
124	H	Me	H	H	H	H	H	H	H	CO <sub>2</sub> Me	H	H
125	H	Me	H	H	H	H	H	H	H	CO <sub>2</sub> H	H	H
126	Me	Me	H	H	H	H	H	H	CH <sub>2</sub> OH	H	H	H
127	H	Me	H	H	H	H	H	H	H	NO <sub>2</sub>	H	H
128	H	Me	H	H	H	H	H	H	H	O   -C-N-   Cyclohexyl	H	H
129	H	Me	H	H	H	H	H	H	H	NH <sub>2</sub>	H	H
130	H	Me	H	H	H	H	H	H	H	CONH <sub>2</sub>	H	H
131	H	Me	H	H	NHAC	H	H	H	H	OMe	H	H

SUBSTITUTE SHEET

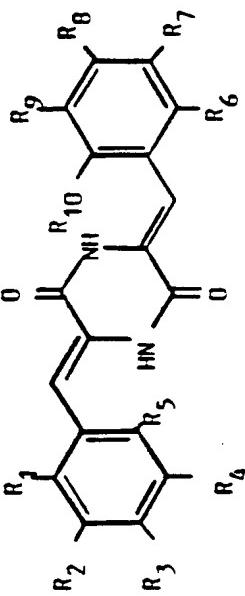
COMPOUND NO.	R <sub>14</sub>	R <sub>15</sub>	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>
132	H	Me	Cl	H	H	Cl	H	H	H	OMe	H	H
133	H	Me	NO <sub>2</sub>	H	H	H	H	H	H	OMe	H	H
134	H	Me	H	H	NMeAC	H	H	H	H	OMe	H	H
135	Me	H	Cl	H	H	Cl	H	H	H	OMe	H	H
136	Me	H	NO <sub>2</sub>	H	H	H	H	H	H	OMe	H	H
137	H	Me	H	H	OMe	H	H	H	H	NHAc	H	H
138	H	Me	-O(CH <sub>2</sub> ) <sub>3</sub> NMe <sub>2</sub> HCl	H	H	H	H	H	H	OMe	H	H
139	Me	Me	H	H	H	H	H	H	H	CF <sub>3</sub>	H	H
140	Me	Me	H	H	H	H	H	H	- benzene -	H	H	H
141	Me	Me	H	H	H	H	H	H	H	NMe <sub>2</sub>	H	H
142	Me	Me	H	H	H	H	OMe	H	H	H	H	H
143	Me	Me	H	H	H	H	H	H	H	NH <sub>2</sub>	H	H
144	Me	Me	F	H	H	H	H	H	H	H	H	H
145	Me	Me	H	F	H	H	H	H	H	H	H	H
146	Me	Me	F	H	F	H	H	H	H	H	H	H
147	Me	Me	H	H	H	H	H	H	H	CH <sub>2</sub> QAC	H	H
148	Me	Me	H	H	H	H	H	CH <sub>2</sub> QAC	H	H	H	H
149	Me	Me	H	H	H	H	H	H	OEt	H	H	H
150	Me	Me	H	H	H	H	H	H	H	H	H	H

COMPOUND NO.	R <sub>14</sub>	R <sub>15</sub>	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>
151	Me	Me	H	H	H	H	H	Cl	H	H	H	Cl
152	H	Me	H	H	OMe	H	H	H	H	NH <sub>2</sub>	H	H
153	Me	Me	H	H	H	H	H	H	H	NMeAc	H	H
154	Me	Me	H	H	H	H	H	H	Cl	Cl	H	H
155	Me	Me	H	H	H	H	H	H	Cl	H	H	H
156	Me	Me	H	H	H	H	H	H	H	SO <sub>2</sub> Me	H	H
157	Me	Me	H	H	H	H	H	H	H	SO <sub>2</sub> NMe <sub>2</sub>	H	H
158	Me	Me	H	H	H	H	H	NMeCOBu <sup>t</sup>	H	H	H	H
159	Me	Me	H	H	H	H	H	H	H	Ph	H	H
160	Me	Me	H	H	H	H	H	H	H	CO <sub>2</sub> Me	H	H
161	Me	Me	H	H	Br	H	H	H	H	H	H	H
162	Me	Me	H	H	OMe	H	H	F	H	F	H	H
163	Me	Me	H	H	OMe	H	H	H	H	Br	H	H
164	Me	Me	H	H	OMe	H	H	H	H	F	H	H
165	Me	Me	Cl	H	OMe	H	Cl	NO <sub>2</sub>	H	H	H	H
166	H	-CH <sub>2</sub> -	H	H	OMe	H	H	H	H	H	H	H
167	H	-CH <sub>2</sub> -	H	H	H	H	H	H	H	OMe	H	H
168	Me	-CH <sub>2</sub> -	H	H	OMe	H	H	H	H	H	H	H
169	Me	Et	H	H	H	H	H	H	H	H	H	H

SUBSTITUTE SHEET

COMPOUND NO.	R <sub>14</sub>	R <sub>15</sub>	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>
170	Me	-CH <sub>2</sub> -C≡	H	H	H	H	H	H	H	OMe	H	H
171	H	Me	H	H	OMe	H	H	H	H	NO <sub>2</sub>	H	H

SUBSTITUTE SHEET

TABLE 2

COMPOUND NO.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>	PREPARED IN REF EXAMPLE
21	C1	H	H		H	C1	H	H	H	H	5
22	H	H	H		H	H	H	H	H	H	10
23	H	H	OAc		H	H	H	H	H	H	6
24	H	H	NO <sub>2</sub>		H	H	H	H	H	H	6
25	H	H	OEt		H	H	H	H	H	H	5
26	H	H	NHAc		H	H	H	OMe	H	H	7
27	H	- Benzene -		H	H	H	H	OMe	H	H	14
28	NO <sub>2</sub>	H	H		H	H	H	OMe	H	H	8
29	C1	H	H		H	C1	H	OMe	H	H	7
30	H	H	NH <sub>2</sub>		H	H	H	H	H	H	13
31	H	OAc	H		H	H	H	H	H	H	6
32	OAc	H	H		H	H	H	H	H	H	6

**SUBSTITUTE SHEET**

COMPOUND NO.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>	PREPARED IN REF EXAMPLE
33	H	OH	H	H	H	H	H	H	H	H	13
34	H	H	NHAC	H	H	H	H	H	H	H	5
35	H	NO <sub>2</sub>	H	H	H	H	NO <sub>2</sub>	H	H	H	11
36	H	H	OH	H	H	H	H	OMe	H	H	13
37	H	H	OAc	H	H	H	H	OMe	H	H	7
38	NHAC	H	H	H	H	H	H	H	H	H	5
39	NH <sub>2</sub>	H	H	H	H	H	H	H	H	H	13
40	H	H	NHAC	H	H	C1	H	H	C1	H	9
41	H	H	NMeAc	H	H	H	H	OMe	H	H	7
42	H	H	C1	H	H	H	H	NHAC	H	H	9
43	H	H	CH <sub>2</sub> OAc	H	H	H	H	H	H	H	5
44	H	H	CH <sub>2</sub> NHAC	H	H	H	H	H	H	H	5
45	H	H	H	H	H	H	H	H	H	H	5
46	H	H	SO <sub>2</sub> Me	H	H	H	H	OMe	H	H	7
47	H	H	OBu <sup>n</sup>	H	H	H	H	OMe	H	H	7
48	H	H	OBu <sup>n</sup>	H	H	H	H	H	H	H	5
49	H	H	OPr <sup>t</sup>	H	H	H	H	OMe	H	H	7
50	H	H	Bu <sup>t</sup>	H	H	H	H	OMe	H	H	7
51	H	H	Bu <sup>t</sup>	H	H	H	H	H	H	H	5

COMPOUND NO.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>	PREPARED IN EXAMPLE
52	H	H	OPr <sup>1</sup>	H	H	H	H	H	H	H	5
53	Br	H	H	H	H	H	H	OMe	H	H	7
54	F	H	F	H	H	H	H	H	H	H	5
55	Br	H	H	H	H	H	H	H	H	H	5
56	H	H	CH <sub>2</sub> NHBOC	H	H	H	H	OMe	H	H	7
57	H	H	OMe	H	H	H	H	CH <sub>2</sub> SMe	H	H	7
58	H	H	NHAC	H	H	H	H	CH <sub>2</sub> OAC	H	H	9
59	H	H	H	H	H	H	H	CH <sub>2</sub> SMe	H	H	5
60	H	H	OMe	H	H	H	H	CH <sub>2</sub> SO <sub>2</sub> Me	H	H	7
61	H	CH <sub>2</sub> SAC	H	H	H	H	H	H	H	H	5
62	H	CO <sub>2</sub> Me	H	H	H	H	H	H	H	H	5
63	H	CH <sub>2</sub> SAC	H	H	H	H	H	OMe	H	H	7
64	H	CH <sub>2</sub> SH	H	H	H	H	H	H	H	H	13
65	H	NO <sub>2</sub>	H	H	H	H	H	H	H	H	6
66	H	H	CH <sub>2</sub> NHBOC	H	H	H	H	H	H	H	5
67	H	H	CH <sub>2</sub> NH <sub>2</sub>	H	H	H	H	OMe	H	H	13
68	H	H	CH <sub>2</sub> NHBOC	H	H	H	H	NHAC	H	H	9
69	F	H	F	H	H	H	H	OMe	H	H	7
70	CF <sub>3</sub>	H	H	H	H	H	H	OMe	H	H	7

COMPOUND NO.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>	PREPARED IN EXAMPLE
71	F	H	H	H	H	H	H	NHAC	H	H	9
72	H	H	F	H	H	H	H	NHAC	H	H	9
73	OMe	H	OMe	H	H	H	H	OMe	H	H	7
74	H	H	NO <sub>2</sub>	H	H	H	H	H	H	H	6
75	H	H	H	H	H	H	H	O(CH <sub>2</sub> ) <sub>2</sub> NMe <sub>2</sub>	H	H	5
76	H	H	H	H	H	H	H	CH <sub>2</sub> SAC	H	H	5
77	F	H	F	H	H	H	H	NHAC	H	H	9
78	CF <sub>3</sub>	H	H	H	H	H	H	NHAC	H	H	9
79	Br	H	H	H	H	H	H	NHAC	H	H	9
80	H	H	OMe	H	H	H	H	CONH <sub>2</sub>	H	H	7
81	H	H	OMe	H	H	H	H	OCOBu <sup>t</sup>	H	H	7
82	H	H	NHAC	H	H	H	H	OCOBu <sup>t</sup>	H	H	9
83	H	H	NHCOOMe	H	H	H	H	OMe	H	H	7
84	C1	H	OH	H	H	H	H	OMe	H	H	7
85	C1	H	OH	H	H	H	H	H	H	H	5
86	H	H	NHAC	H	H	H	H	NMe <sub>2</sub>	H	H	12
87	H	H	NHCOCH <sub>2</sub> OAc	H	H	H	H	OMe	H	H	7
88	H	H	NHCOCH <sub>2</sub> OH	H	H	H	H	OMe	H	H	13
89	H	H	H	H	H	-Benzene-	H	NMe <sub>2</sub>	H	H	5

COMPOUND NO.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>	PREPARED IN EXAMPLE
90	H	OMe	OMe	H	H	H	H	H	H	H	5
91	H	OMe	OMe	H	H	H	H	OMe	H	H	7
92	H	OMe	OMe	H	H	H	H	NHAC	H	H	9
93	H	H	OCH <sub>2</sub> CO <sub>2</sub> Me	H	H	H	H	H	H	H	5
94	H	H	CH <sub>2</sub> NHCO(CH <sub>2</sub> ) <sub>3</sub> CO <sub>2</sub> Me	H	H	H	H	H	H	H	5
95	H	H	CH <sub>2</sub> NHCO(CH <sub>2</sub> ) <sub>4</sub> CO <sub>2</sub> Et	H	H	H	H	H	H	H	5
96	H	H	O(CH <sub>2</sub> ) <sub>4</sub> CO <sub>2</sub> Me	H	H	H	H	H	H	H	5
97	H	H	O(CH <sub>2</sub> ) <sub>4</sub> CO <sub>2</sub> H	H	H	H	H	H	H	H	13
98	H	H	O(CH <sub>2</sub> ) <sub>1</sub> NMe <sub>2</sub> .HCl	H	H	H	H	H	H	H	15
99	H	H	O(CH <sub>2</sub> ) <sub>2</sub> NMe <sub>2</sub> .HCl	H	H	H	H	H	H	H	15
100	H	H	CH <sub>2</sub> NHCO(CH <sub>2</sub> ) <sub>1</sub> CO <sub>2</sub> Me	H	H	H	H	OMe	H	H	7
101	H	H	OCH <sub>2</sub> CO <sub>2</sub> H	H	H	H	H	H	H	H	13
102	H	H	O(CH <sub>2</sub> ) <sub>2</sub> NMe <sub>2</sub>	H	H	H	H	H	H	H	5
103	F	H	H	H	H	H	H	OMe	H	H	7
104	H	H	CH <sub>2</sub> OH	H	H	H	H	NHAC	H	H	13
105	H	H	H	H	H	H	H	CN	H	H	6

- 30 -

Cancer cells which exhibit multiple drug resistance, referred to as MDR cells, display a reduction in intracellular drug accumulation compared with the corresponding drug-sensitive cells. Studies using in vitro 5 derived MDR cell lines have shown that MDR is often associated with increased expression of a plasma membrane glycoprotein (P-gp) which has drug binding properties. P-gp is thought to function as an efflux pump for many hydrophobic compounds, and transfection studies using 10 cloned P-gp have shown that its overexpression can confer the MDR phenotype on cells: see, for example, Ann. Rev. Biochem 58 137-171 (1989).

A major function of P-gp in normal tissues is to export intracellular toxins from the cell. There is 15 evidence to suggest that overexpression of P-gp may play a clinical role in multiple drug resistance. Increased levels of P-gp mRNA or protein have been detected in many forms of human cancers - leukaemias, lymphomas, sarcomas and carcinomas. Indeed, in some cases P-gp levels have 20 been found to increase in tumour biopsies obtained after relapse from chemotherapy.

Inhibition of P-gp function in P-gp mediated MDR has been shown to lead to a net accumulation of anti-cancer agent in the cells. For example, Verapamil a known calcium 25 channel blocker was shown to sensitise MDR cells to vinca alkaloids in vitro and in vivo: Cancer Res., 41, 1967-1972 (1981). The proposed mechanism of action involves competition with the anti-cancer agent for binding to the P-gp. A range of structurally unrelated resistance-modifying agents acting by this mechanism have been 30 described such as tamoxifen (Nolvadex:ICI) and related compounds, and cyclosporin A and derivatives.

Compounds of formula A, both novel and known, and their pharmaceutically acceptable salts and esters 35 (hereinafter referred to as "the present compounds") have been found in biological tests to have activity in modulating multiple drug resistance. The results are set

- 31 -

- out in Example 15 which follows. The present compounds may therefore be used as multiple drug resistance modifying agents, also termed resistance-modifying agents, or RMAs. The present compounds can modulate, e.g. reduce, or
- 5 eliminate multiple drug resistance. The present compounds can therefore be used in a method of potentiating the cytotoxicity of an agent which is cytotoxic to a tumour cell. Such a method comprises, for instance, administering one of the present compounds to the tumour cell whilst the
- 10 tumour cell is exposed to the cytotoxic agent in question. The therapeutic effect of a chemotherapeutic, or antineoplastic, agent may thus be enhanced. The multiple drug resistance of a tumour cell to a cytotoxic agent during chemotherapy may be reduced or eliminated.
- 15 A human or animal patient harbouring a tumour may be treated for resistance to a chemotherapeutic agent by a method comprising the administration thereto of one of the present compounds. The present compound is administered in an amount effective to potentiate the cytotoxicity of the
- 20 said chemotherapeutic agent. Examples of chemotherapeutic or antineoplastic agents which are preferred in the context of the present invention include vinca alkaloids such as vincristine and vinblastine; anthracycline antibiotics such as daunorubicin and doxorubicin; mitoxantrone; actinomycin
- 25 D and plicamycin.

The present compounds can be administered in a variety of dosage forms, for example orally such as in the form of tablets, capsules, sugar- or film-coated tablets, liquid solutions or suspensions or parenterally, for

30 example intramuscularly, intravenously or subcutaneously. The present compounds may therefore be given by injection or infusion.

The dosage depends on a variety of factors including the age, weight and condition of the patient and the route

35 of administration. Typically, however, the dosage adopted for each route of administration when a compound of the invention is administered alone to adult humans is 0.001 to

10 mg/kg, most commonly in the range of 0.01 to 5 mg/kg, body weight. Such a dosage may be given, for example, from 1 to 5 times daily by bolus infusion, infusion over several hours and/or repeated administration.

5 A diketopiperazine of formula (A) or a pharmaceutically acceptable salt or ester thereof is formulated for use as a pharmaceutical or veterinary composition also comprising a pharmaceutically or 10 veterinarily acceptable carrier or diluent. The compositions are typically prepared following conventional methods and are administered in a pharmaceutically or 15 veterinarily suitable form. An agent for use as a modulator of multiple drug resistance comprising any one of the present compounds is therefore provided.

15 For example, the solid oral forms may contain, together with the active compound, diluents such as lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants such as silica, talc, stearic acid, magnesium or calcium stearate and/or polyethylene 20 glycols; binding agents such as starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose, or polyvinyl pyrrolidone; disintegrating agents such as starch, alginic acid, alginates or sodium starch glycolate; effervescent mixtures; dyestuffs, sweeteners; wetting 25 agents such as lecithin, polysorbates, lauryl sulphates. Such preparations may be manufactured in known manners, for example by means of mixing, granulating, tabletting, sugar coating, or film-coating processes.

Liquid dispersions for oral administration may be 30 syrups, emulsions and suspensions. The syrups may contain as carrier, for example, saccharose or saccharose with glycerol and/or mannitol and/or sorbitol. In particular, a syrup for diabetic patients can contain as carriers only products, for example sorbitol, which do not metabolise to 35 glucose or which only metabolise a very small amount to glucose. The suspensions and the emulsions may contain as carrier, for example, a natural gum, agar, sodium alginate,

- 33 -

pectin, methylcellulose, carboxymethylcellulose or polyvinyl alcohol.

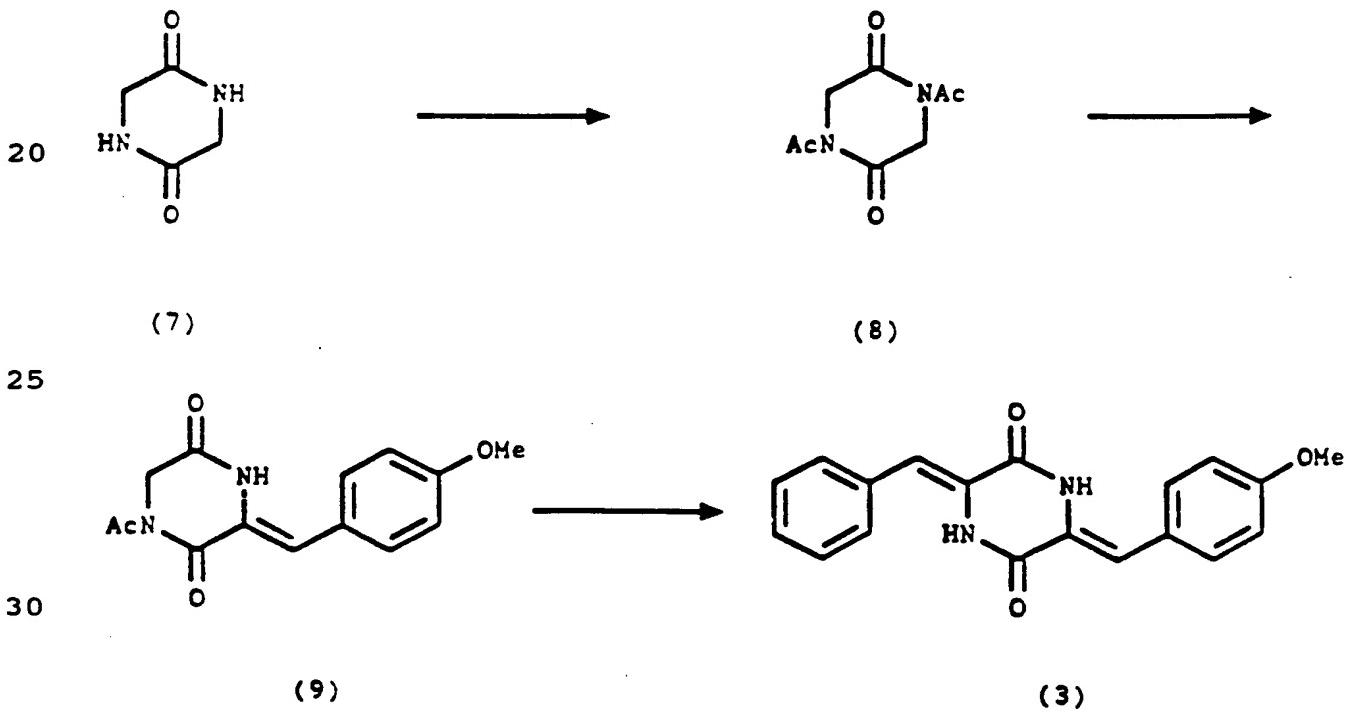
Suspensions or solutions for intramuscular injections may contain, together with the active compound, a

5 pharmaceutically acceptable carrier such as sterile water, olive oil, ethyl oleate, glycols such as propylene glycol, and, if desired, a suitable amount of lidocaine hydrochloride. Some of the present compounds are insoluble in water. A compound may be encapsulated within liposomes.

10

The following Examples illustrate the invention:

Reference Example 1: Preparation of  
(3Z,6Z)-6-Benzylidene-3-(4-methoxybenzylidene)-2,5-  
15 piperazinedione (3) (scheme 1)



35 1,4-Diacetyl-2,5-piperazinedione (8)

1,4-Diacetyl-2,5-piperazinedione (8) was prepared by the published procedure (S.M. Marcuccio and J.A. Elix,

- 34 -

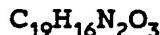
Aust. J. Chem., 1984, 37, 1791).

(3Z)-1-Acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione  
(9)

(3Z)-1-Acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (9) was prepared by the published procedure (T. Yokoi, L-M. Yang, T. Yokoi, R-Y. Wu, and K-H. Lee, J. Antibiot., 1988, 41, 494).

10 (3Z,6Z)-6-Benzylidene-3-(4-methoxybenzylidene)-2,5-piperazinedione (3)

A mixture of (3Z)-1-acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (9) (1.0g, 3.6 mmol), benzaldehyde (430  $\mu$ l, 4.2 mmol) and triethylamine (1.14 ml), 8.2 mmol), in dry DMF (20 ml), was heated at 130°C for 18h. The reaction mixture was cooled to room temperature and poured into ethyl acetate (100 ml). A yellow solid precipitated which was filtered off and dried. Yield 360 mg (31%).



20  $^1\text{H}$  nmr (400 MHz  $\text{d}_6$ -DMSO):

$\delta$ : 3.80 (3H, s, O-Me); 6.77 (1H, s, CH=C);  
 6.78 (1H, s, CH=C); 6.98 (2H, d, J=8Hz, 2xC-H on Ar-  
 OMe); 7.30-7.56 (7H, m, Ph and 2xC-H on Ar-OMe);  
 10.15 (2H, br.s, N-H).

25  $^{13}\text{C}$  nmr (100 MHz  $\text{d}_6$ -DMSO)

6: 58.68; 117.66; 118.03; 118.77; 128.11; 128.92;  
129.95; 131.53; 132.11; 132.69; 134.44; 136.59;  
161.39; 161.62; 162.71.

ms (desorption chemical ionisation, ammonia):

30 m/z (% relative intensity) : 321 (100)  $MH^+$ .

ir : KBr (diffuse reflectance):

$\nu$  max ( $\text{cm}^{-1}$ ) : 1620, 1700, 3100, 3220.

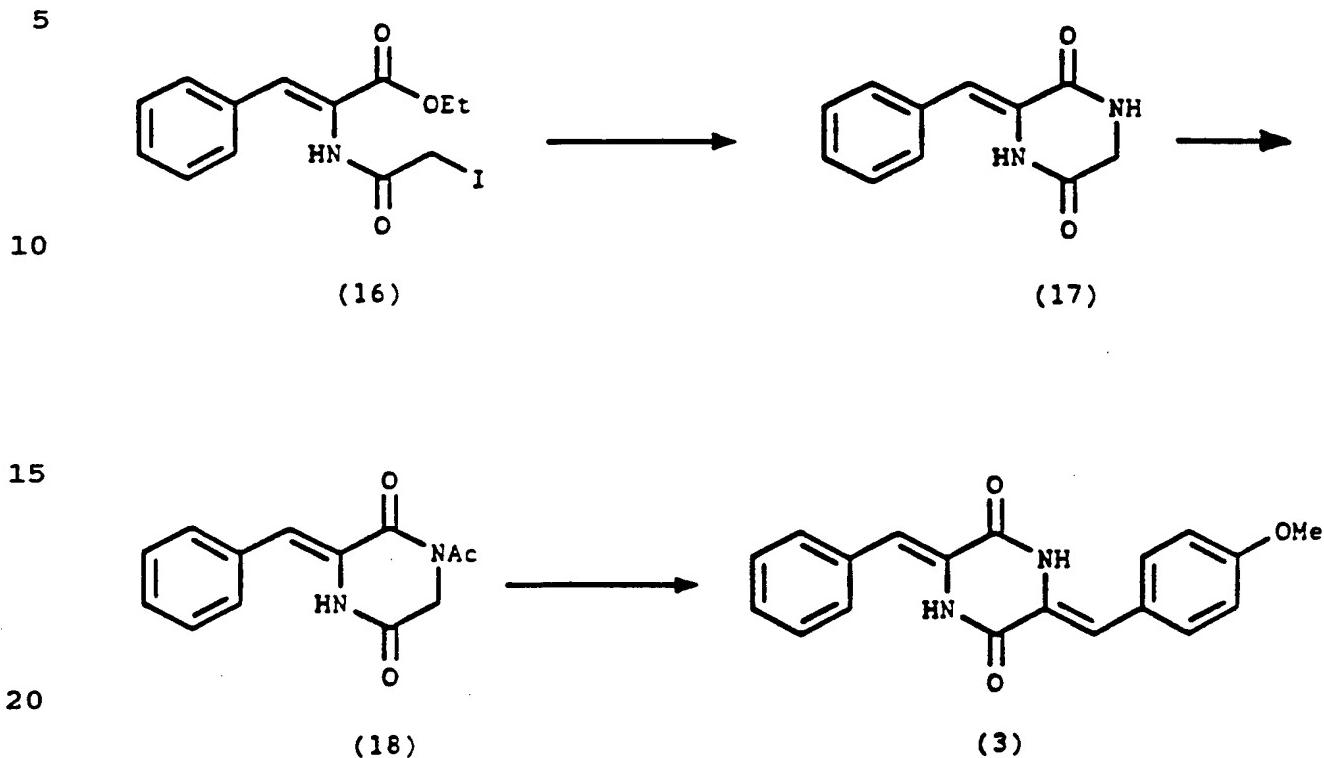
#### **Elemental analysis:**

Calculated for C<sub>19</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>: C 71.24, H 5.03, N 8.74.

35 Found: C 70.92, H 5.02, N 8.80.  
C 70.89, H 5.06, N 8.79%

- 35 -

Reference Example 2: Preparation of (3Z,6Z)-6-Benzylidene-3-(4-methoxybenzylidene)-2,5-piperazinedione (3) (Scheme 2)



Compound 16 is treated with ammonia and subsequently  
25 with acetic anhydride to yield 1-acetyl-3-benzylidene-2,5-  
piperazinedione (18).

Compound 18 is then condensed, in the presence of  
caesium carbonate or triethylamine in DMF, with 4-  
methoxybenzaldehyde to yield compound 3.

30

Reference Example 3: Preparation of 1-acetyl-3-benzylidene-2,5-piperazinedione

1,4-Diacetyl-2,5-piperazinedione (25.0g, 126 mmol),  
35 which is compound (8) mentioned in Reference Example 1, was  
heated at 120-130°C in DMF (200 ml) with triethylamine  
(17.6 ml, 126 mmol) and benzaldehyde (13.0 ml, 126 mmol).

- 36 -

After 4 h the mixture was cooled to room temperature and poured into EtOAc (1000 ml), and washed three times with brine. Any solid formed at this stage was filtered off. The filtrate was dried ( $MgSO_4$ ) and the solvent removed in 5 vacuo. The residue was recrystallised from EtOAc:Hexane to give 11.78 g (38%) of the title compound as a yellow solid.

<sup>1</sup>H NMR ( $CDCl_3$ , 400 MHz)  $\delta$ =2.69 (3H, s) 4.54 (2H, s) 7.20 (1H, s) 7.40 (3H, m), 7.48 (2H, m), 7.93 (1H, br.s)

10 MS (DCI,  $NH_3$ ): 262 ( $MNH_4^+$ , 20%), 245 ( $MH^+$ , 53%), 220 (52%), 204 (100%), 203 (100%)

Microanalysis		C	H	N
Calc		63.93	4.95	11.47
Found		64.11	5.02	11.41
		64.05	4.90	11.44

Reference Example 4: Preparation of 1-acetyl-3-(4-acetamidobenzylidene)-2,5-piperazinedione

1,4-Diacetyl-2,5-piperazinedione (10.0g, 50 mmol), prepared by the published procedure mentioned in Reference Example 1, was stirred in DMF (40 ml) with 4-acetamidobenzaldehyde (8.24 g, 50 mmol) and triethylamine 25 (7 ml, 50 mmol) and heated to 120°C. After 2½ h the mixture was cooled to room temperature, diluted with EtOAc (100 ml) and stirred overnight. The solid formed was collected, washed with EtOAc and dried to give 8.46 g (56%) of a yellow solid.

30 <sup>1</sup>H NMR ( $CDCl_3+CF_3CO_2H$ , 400 MHz)  $\delta$ =2.32 (3H, s) 2.72 (3H, s) 4.68 (2H, s) 7.36 (1H, s) 7.45 (2H, d,  $J=8Hz$ ) 7.60 (2H, d,  $J=8Hz$ )

Microanalysis		C	H	N
Calc		59.80	5.02	13.95
Found		60.08	5.09	13.89

- 37 -

	60.11	5.07	13.86
--	-------	------	-------

Reference Example 5: Preparation of compound 96

5        1-Acetyl-3-benzylidene-2,5-piperazinedione (one equivalent), prepared according to Reference Example 3, was treated with 5-(4-formylphenoxy)pentanoic acid, methyl ester in the presence of  $\text{Cs}_2\text{CO}_3$  (1-1.1 equivalents) in DMF at 80-100°C for 1-8 hours. The title compound was obtained  
10      in 39% yield.

By the same method, but replacing 5-(4-formylphenoxy)pentanoic acid, methyl ester (which is benzaldehyde substituted at position 4 by  $-\text{O}(\text{CH}_2)_4\text{CO}_2\text{Me}$ ) by the appropriately substituted benzaldehyde, the following  
15      compounds were prepared:

Compound	Yield (%)	Compound	Yield (%)
21	66	25	37
34	56	89	37
38	84	43	54
40	44	45	91
44	44	51	68
48	69	54	69
52	72	59	50
55	73	62	63
61	44	75	49
66	15	85	15
76	60	90	74
89	37	94	39
93	69	96	39
95	26		
102	45		

Reference Example 6: Preparation of Compound 31

1-Acetyl-3-benzylidene-2,5-piperazinedione (one equivalent), prepared according to Reference Example 1, was  
35      treated with 3-acetoxybenzaldehyde (one equivalent) in the

- 38 -

presence of triethylamine (1-2 equivalents) in DMF at 130°C for 2-6 hours. The title compound was obtained in 61% yield.

- By the same method, but replacing 3-
- 5 acetoxybenzaldehyde by the appropriately substituted benzaldehyde, the following compounds were prepared:

	Compound	Yield (%)
10	23	16
	24	43
	32	41
	65	27
	74	77
15	105	50

**Reference Example 7: Preparation of compound 103**

- 1-Acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (1 equivalent), which is compound (9) mentioned in
- 20 Reference Example 1, was treated with 2-fluorobenzaldehyde (1 equivalent) in the presence of Cs<sub>2</sub>CO<sub>3</sub> (1-1.1 equivalents) in DMF at 80-100°C for 1-6 hours. The title compound was obtained in 69% yield.

- By the same method, but replacing the 2-
- 25 fluorobenzaldehyde by the appropriately substituted benzaldehyde with the exception of compound 84 which was prepared by condensation with 4-acetoxy-2-chlorobenzaldehyde, the following compounds were prepared:

30	Compound	Yield (%)	Compound	Yield (%)	
	26	80		63	71
	29	70		69	20
	37	21		70	10
35	41	34		73	38
	46	16		80	45

- 39 -

	47	46		81	5
	49	60		83	41
	50	56		84	Low
	53	77		87	33
5	57	49		91	74
	60	71		100	20
				103	69

Reference Example 8: Preparation of compound 28

10        1-Acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (1 equivalent), compound (9) in Reference Example 1, was treated with 2-nitrobenzaldehyde (1 equivalent) and triethylamine (1-2 equivalents) and DMF at 130°C for 2-6 hours. The title compound was obtained in 45% yield.

15

Reference Example 9: Preparation of Compound 77

16        1-Acetyl-3-(4-acetamidobenzylidene)-2,5-piperazinedione (1 equivalent), prepared according to Reference Example 4, was treated with 2,4-difluorobenzaldehyde (1 equivalent) in the presence of Cs<sub>2</sub>CO<sub>3</sub> (1-1.1 equivalents) in DMF at 80-100°C for 1-6 hours. The title compound was obtained in 60% yield.

20

17        By the same method, but replacing 2,4-difluorobenzaldehyde by the appropriately substituted benzaldehyde, the following compounds were obtained:

25

Compound	Yield (%)	Compound	Yield (%)
42	50	40	40
68	26	58	22
72	41	71	36
79	11	78	16
92	68	82	16

Reference Example 10: Preparation of compound 22

30        1,4-Diacetyl-2,5-piperazinedione (1 equivalent), prepared by the published procedure mentioned in Reference

- 40 -

Example 1, was treated with benzaldehyde (2.1 equivalents) in the presence of triethylamine (2.5 equivalents) in DMF at 130°C for 8 hours. The title compound was obtained in 89% yield.

5

Reference Example 11: Preparation of compound 35

1,4-Diacetyl-2,5-piperazinedione (1 equivalent), prepared by the published procedure mentioned in Reference Example 1, was treated with 3-nitrobenzaldehyde (1 equivalent) in the presence of triethylamine (1 equivalent) in DMF at room temperature for 18-20 hrs. The title compound was obtained in 9% yield together with 1-acetyl-3-(3-nitrobenzylidene)-2,5-piperazinedione (66% yield).

15    Reference Example 11a: Preparation of 1-acetyl-3-(4-dimethylaminobenzylidene)-2,5-piperazinedione

1,4-Diacetyl-2,5-piperazinedione, (1 equivalent), prepared as described in Reference Example 1, was treated with 4-dimethylaminobenzaldehyde (1 equivalent) in the presence of Et<sub>3</sub>N in DMF at 130°C for 24 hrs. The title compound was obtained in 18% yield

Reference Example 12: Preparation of Compound 86

25    1-Acetyl-3-(4-dimethylaminobenzylidene)-2,5-piperazinedione (1 equivalent) as described in Reference Example 11a was reacted with 4-acetamidobenzaldehyde (1 equivalent) in the presence of Cs<sub>2</sub>CO<sub>3</sub> (1 equivalent) in DMF at 80°C for 2-6 hours. The title compound was obtained in 30 56% yield.

Reference Example 13: Interconversions of Reference compounds

(i) Compound 31, prepared as described in Reference Example 6, was treated with aqueous lithium hydroxide in a mixture of MeOH and THF at room temperature for 2-3 hrs to give compound 33 in 91% yield.

- 41 -

- (ii) Compound 61, prepared as described in Reference Example 5, was treated with aqueous lithium hydroxide in a mixture of MeOH and THF at room temperature for 3 hours to give compound 64 in 57% yield.
- 5 (iii) Compound 96, prepared as described in Reference Example 5, was treated with aqueous sodium hydroxide in THF at room temperature for 4 hours to give compound 97 in 54% yield.
- (iv) Compound 37, prepared as described in Reference
- 10 Example 7, was treated with aqueous sodium hydroxide in THF at room temperature for 8 hrs to give compound 36 in 30% yield.
- (v) Compound 56, prepared as described in Reference Example 7, was treated with trifluoroacetic acid in CH<sub>2</sub>Cl<sub>2</sub>,
- 15 at room temperature for 12 hrs to give compound 67 in 96% yield.
- (vi) Compound 87, prepared as described in Reference Example 7, was treated with aqueous sodium hydroxide in THF at room temperature for 4 hours to give compound 88 in 69% yield.
- 20 (vii) Compound 65, prepared as described in Reference Example 6 was hydrogenated over 10% palladium on carbon as catalyst in CH<sub>2</sub>Cl<sub>2</sub>, in the presence of a few drops of trifluoroacetic acid to give compound 39 in 38% yield.
- 25 Under the same conditions of hydrogenation compound 74 was converted into compound 30 in 95% yield.
- (viii) Compound 93, prepared as described in Reference Example 5, was hydrolysed by treatment with aqueous sodium hydroxide in a mixture of MeOH and THF at room temperature
- 30 for 18 hours to give compound 101 in 72% yield.
- (ix) Compound 58, prepared as described in Example 9, was hydrolysed by treatment with aqueous sodium hydroxide in THF at room temperature for 3 hours to give compound 104 in 90% yield.

35

Reference Example 14: Preparation of Compound 27

1-Acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (1

- 42 -

equivalent), compound (9) in Reference Example 1, was treated with 2-naphthaldehyde (1 equivalent) in the presence of  $\text{Cs}_2\text{CO}_3$  (1.0-1.1 equivalents) in DMF at 80-100°C for 1-6 hours. The title compound was obtained in 84%  
5 yield.

Reference Example 15:

Preparation of Salts

Compound 98, the hydrochloride salt of compound 102,  
10 was prepared by treatment of a solution of compound 102 in THF with 2 molar hydrochloric acid followed by sonication until a clear solution was obtained. The solvent was then removed in vacuo and the residual solution was freeze-dried to give compound 98.

15 Compound 99 was prepared by bubbling HCl gas through a solution of the corresponding free base in THF, followed by evaporation to dryness.

- 43 -

Reference Example 16: Preparation of (3Z)-1-acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (9) and 1-acetyl-3-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione (10) (Scheme 3)

5



10

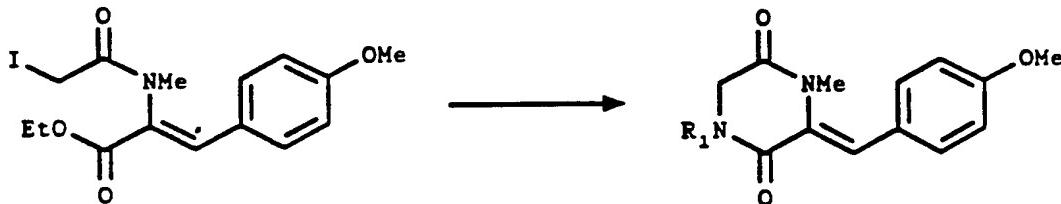
(12)

(13)  $R_1 = H$ (9)  $R_1 = Ac$ 

15



20



25

(14)

(15)  $R_1 = H$ (10)  $R_1 = Ac$ 

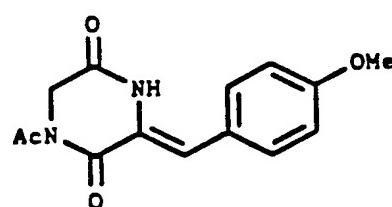
Compound 12 is treated with  $NH_3$  to afford 3-(4-methoxybenzylidene)-2,5-piperazinedione (13). This is then treated with acetic anhydride to yield (3Z)-1-acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (9).

Compound 12 is treated with, as methylating agent, iodomethane in the presence of potassium carbonate in dimethylformamide to give compound 14. Compound 14 is then treated with  $NH_3$  and subsequently with acetic anhydride to yield 1-acetyl-3-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione (10).

- 44 -

EXAMPLE 1: Preparation of (3Z,6Z)-3-benzylidene-6-(4-methoxybenzylidene)-1-methyl-2,5-piperazine dione (1) (Scheme 4)

5

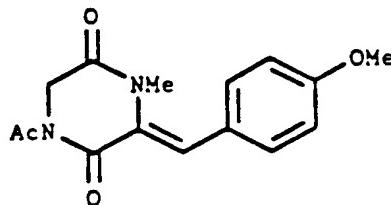


10

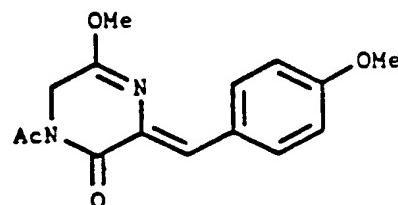
(9)

15

20



+

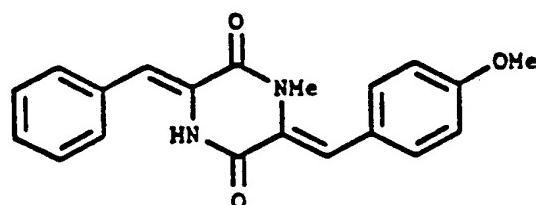


25

(10)

(11)

30



35

(1)

- 45 -

(3Z)-1-Acetyl-3-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione (10) and  
1-Acetyl-5-methoxy-3-(4-methoxybenzylidene)-3,6-dihydropyrazin-2-one (11)

5 A mixture of

(3Z)-1-Acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (9) (2.0g, 7.3 mmol), methyl iodide (0.46 ml, 7.3 mmol), and sodium carbonate (800 mg, 7.5 mmol) in dry DMF (50 ml) was stirred under an atmosphere of dry nitrogen for 3 days.

10 The reaction mixture was then poured into ethyl acetate (500 ml) and washed with water (4x100 ml) and brine. The organic phase was separated, dried ( $\text{MgSO}_4$ ), and the solvent removed in vacuo. The residue was purified by flash chromatography (silica, EtOAc:Hexane, 1:1) to give

15 (3Z)-1-acetyl-3-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione (10) 1.38 g (66%) as a yellow solid and 1-acetyl-5-methoxy-3-(4-methoxybenzylidene)-3,6-dihydropyrazin-2-one (11) 248 mg (11.8%) as a bright yellow solid.

20 (3Z)-1-Acetyl-3-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione (10):

$\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_4$

$^1\text{H}$  nmr (400 MHz  $\text{CDCl}_3$ ):

25  $\delta$ : 2.63 (3H, s, Ac); 2.95 (3H, s, N-Me); 3.87 (3H, s, O-Me); 4.52 (s, 2H, N- $\text{CH}_2$ -CO); 6.93 (2H, d,  $J=8\text{Hz}$ , Aromatic); 7.26 (1H, s, C=CH); 7.29 (2H, d,  $J=8\text{Hz}$ ), Aromatic).

ms (desorption chemical ionisation, ammonia):

m/z (% relative intensity): 306 (34%)  $\text{MNH}_4^+$ ; 289 (100%);

30 216 (14%)

ir : KBr (diffuse reflectance)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1690, 1700, 3000.

Elemental analysis:

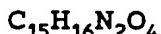
Calculated for  $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_4$ : C 62.49, H 5.59, N 9.72

35 C 62.48, H 5.58, N 9.68.  
C 62.51, H 5.65, N 9.67%

1-Acetyl-5-methoxy-3-(4-methoxybenzylidene)-3,6-

- 46 -

dihydropyrazin-2-one (11):



$^1H$  nmr (400 MHz CDCl<sub>3</sub>):

δ: 2.68 (3H, s, Ac); 3.86 (3H, s, Ar-OMe); 3.99 (3H, s, O-Me); 4.44 (s, 2H, N-CH<sub>2</sub>-CO); 6.95 (2H, d, J=8Hz, Ar); 7.32 (1H, s, C=CH); 8.03 (2H, d, J=8Hz, Ar).

ms (desorption chemical ionisation, ammonia):

m/z (% relative intensity): 289 (100%) MH<sup>+</sup>; 247 (14%)

ir : KBr (diffuse reflectance):

10  $\nu_{max}$  (cm<sup>-1</sup>): 1610, 1690, 1700, 1740, 2950.

Elemental Analysis:

Calculated for C<sub>15</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>: C 62.49, H 5.59, N 9.72.

C 62.52, H 5.59, N 9.64.

C 62.52, H 5.64, N 9.66%

15

(3Z,6Z)-3-Benzylidene-6-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione (1)

A mixture of

(3Z)-1-Acetyl-3-(4-methoxybenzylidene)-4-methyl-2,5-

20 piperazinedione (10) (200 mg, 0.69 mmol) and sodium hydride (60% dispersion in oil, 28 mg, 0.69 mmol) in dry DMF (10 ml) was stirred at room temperature for 18 h. Benzaldehyde (71μl, 0.69 mmol) was then added and the reaction mixture stirred at room temperature for 18h. It was then diluted 25 with ethyl acetate (100 ml) and washed with brine (4 x 50 ml). The organic phase was separated, dried (MgSO<sub>4</sub>), and the solvent removed in vacuo. The residue was purified by flash chromatography (silica, dichloromethane containing 1% MeOH) to give 48 mg (21%) of a yellow solid.

30 C<sub>20</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>

$^1H$  nmr (400 MHz CDCl<sub>3</sub>):

δ: 3.06 (3H, s, N-Me); 3.87 (3H, s, O-Me); 6.93 (2H, d, J=8Hz, 2xC-H on Ar-OMe); 7.06 (1H, s, Ph-CH=C); 7.23 (2H, d, J=8Hz, 2xC-H on Ar-OMe); 7.27 (1H, s, MeOAr-CH=C); 7.30-7.48 (5H, m, Ph); (1H, br.s, N-H).

$^{13}C$  nmr (100 MHz CDCl<sub>3</sub>)

δ: 36.62; 55.34; 113.86; 116.80; 121.30; 126.02; 126.14;

- 47 -

128.47; 128.78; 129.06; 129.45; 131.11; 133.07; 159.66;  
159.68; 159.95.

ms (desorption chemical ionisation, ammonia) : 335 (100%)  
 $\text{MH}^+$ .

5 ir : KBr (diffuse reflectance) :  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ) : 1690, 3000,  
3180, 3400.

Elemental analysis:

Calculated for  $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_3$ : O 71.84, H 5.43, N 8.38.

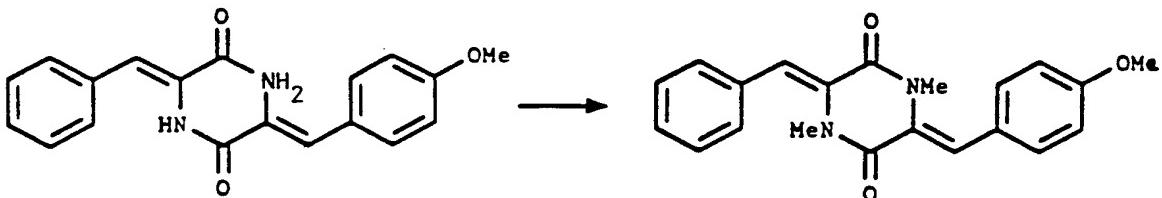
C 71.81, H 5.31, N 8.31.

10 C 71.80, H 5.25, N 8.31%.

EXAMPLE 2: Preparation of (3Z,6Z)-3-benzylidene-6-(4-methoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione (2) (Scheme 5)

15

20



(1)  $\text{R}_2 = \text{Me}$

(2)

(3)  $\text{R}_2 = \text{H}$

25

(3Z,6Z)-3-Benzylidene-6-(4-methoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione (2)

A mixture of

30 (3Z,6Z)-3-Benzylidene-6-(4-methoxybenzylidene)-2,5-piperazinedione (3) (0.5 g, 1.56 mmol), sodium hydride (60% dispersion in mineral oil, 125 mg, 3.1 mmol) and methyl iodide (243  $\mu\text{l}$ , 3.9 mmol) in dry DMF (50 ml) was stirred at room temperature for 4 days. The solvent was then removed

35 in vacuo and the residue purified by flash chromatography (silica, eluting with EtOAc:Hexane, 1:3) to give 220 mg (40%) of compound 2 as a yellow solid.

- 48 -



<sup>1</sup>H nmr (400 MHz CDCl<sub>3</sub>)

δ: 2.95 (3H, s, N-Me); 3.04 (3H, s, N-Me); 3.85 (3H, s, O-Me); 6.90 (2H, d, J=8Hz, 2xC-H on Ar-OMe); 7.19 (1H, s, CH=C); 7.21 (1H, s, CH=C) 7.30-7.56 (7H, m, Ph and 2xC-H on Ar-OMe).

ms (desorption chemical ionisation, ammonia) :

m/z (% relative intensity): 349 (100) MH<sup>+</sup>.

10 **Example 3: Preparation of Compound 146**

Compound 54 (1 equivalent), prepared as in reference Example 5, was treated with sodium hydride (2.0-3.5 equivalents) in DMF at 0°C for 10-90 minutes. The resulting solution was reacted with methyl iodide (2.0-5.0 equivalents) in DMF at room temperature for 3-48 hours. The solvent was then removed in vacuo and the residue purified by flash chromatography to give compound 146 in 49% yield.

By an analogous process other compounds of formula A were prepared by replacing starting compound 54 by the appropriate N-unsubstituted 1,3-dibenzylidene-2,5-piperazinedione bearing the desired substitution pattern in the aromatic ring of one of the benzylidene groups, for instance any of the compounds prepared in the Reference Examples. The following compounds were prepared in this way.

Compound	Yield (%)	Compound	Yield (%)
139	31	141	50
142	50	143	41
144	41	145	51
147	94	149	42
150	43	151	54
153	26	154	71
155	42	156	42
157	24	159	

- 49 -

160	13	161	13
-----	----	-----	----

By methylating (*3Z,6Z*)-6-(2,6-dichlorobenzylidene)-3-(2-nitrobenzylidene)-2,5-piperazinedione under analogous  
5 conditions compound 165 was obtained in 23% yield.  
Compound 153 was prepared from compound 34.

Characterising data for the prepared compounds are provided in Example 16.

10 **Example 4: Preparation of compound 162**

Compound 69 (1 equivalent), prepared as in Reference Example 7, was treated with sodium hydride (2.0-3.5 equivalents) in DMF at 0°C for 10-90 minutes. The resulting solution was reacted with methyl iodide (2.0-5.0 equivalents) in DMF at room temperature for 3-48 hours. The solvent was then removed in vacuo and the residue purified by flash chromatography to give compound 162 in 13% yield. By an analogous process compounds 163 and 164 were prepared.

20

Compound	Yield (%)
163	9
164	20

25 Characterising data for the compounds are set out in Example 16.

**Example 5: Preparation of compound 131**

1-Acetyl-3-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione (1 equivalent), compound 10 described in Example 1, was treated with 4-acetamidobenzaldehyde (1 equivalent), and Cs<sub>2</sub>CO<sub>3</sub> (1.0-1.1 equivalents) in dimethylformamide at 80°C-100°C for 2-5 hours. Compound 131 was obtained in 48% yield.

35 By an analogous process other compounds of formula

- 50 -

(A) were prepared by replacing 4-acetamidobenzaldehyde by the appropriately substituted benzaldehyde which will lead to the desired substitution pattern in ring a of the final product. The following compounds were prepared in this  
5 way.

	<b>Compound</b>	<b>Yield (%)</b>
10	132	56
	133	34
	134	67

Characterising data for the compounds are set out in Example 16.

15

**Example 6: Preparation of compounds 121, 122 and 170**

1-Acetyl-3-benzylidene-2,5-piperazinedione (compound 18 prepared in Reference Example 2) was N-methylated by treatment with methyl iodide (2.0 equivalents) in the  
20 presence of Na<sub>2</sub>CO<sub>3</sub> (1.0 equivalents) in DMF at room temperature for 24 hours. The solvent was removed and the residue purified by flash chromatography. The product 1-acetyl-3-benzylidene-4-methyl-2,5-piperazinedione, obtained in 45% yield, was treated with 4-methoxybenzaldehyde (1.0-  
25 1.1 equivalents) in the presence of Cs<sub>2</sub>CO<sub>3</sub> in DMF at 80°C for 206 hours to give compound 121 in 35% yield.

By an analogous method, but using benzaldehyde in place of 4-methoxybenzaldehyde, compound 122 was obtained in 68% yield.

30 Compound 121 prepared as described above was treated with sodium hydride (1.1 equivalents) and bromomethylcyclopropane (1.0 equivalents) in DMF at room temperature for 4 days to give compound 170 in 9% yield.

Characterising data are provided in Example 16.

35

**Example 7: Preparation of Compounds 124, 125, 128 and 130**

4-((3Z)-1-acetyl-2,5-dioxopiperazin-3-

- 51 -

ylidene)methylbenzoic acid, methyl ester (1 equivalent) was alkylated at the nitrogen at position 4 of the piperazine ring by treatment with methyl iodide (2.0 equivalents) in the presence of  $\text{Na}_2\text{CO}_3$  in DMF for 48 hours. The solvent 5 was removed and the residue purified by flash chromatography. The resulting compound (1 equivalent), obtained in 51% yield, was treated with benzaldehyde (1.0 equivalents) in DMF in the presence of  $\text{Cs}_2\text{CO}_3$  (1.1 equivalents) at 90°C for 2-5 hours to give compound 124 in 10 54% yield.

By hydrolysing compound 124 with aqueous NaOH in methanol and tetrahydrofuran at room temperature for 5 hours, compound 125 was obtained in 79% yield.

Compound 125 (1 equivalent) was treated with  $\text{EtOCOCl}$  15 (1 equivalent) and triethylamine (1 equivalent) in  $\text{CH}_2\text{Cl}_2$  at 0°C for 30 minutes. Treatment of the resultant solution with ammonia then gave compound 130 in 82% yield. Alternatively, treatment with pyrrolidine (1.0 equivalents) gave compound 128 in 39% yield.

20 Characterising data for the compounds are provided in Example 16.

Example 8:      Preparation of compounds 127, 129, 137, 152 and 171

25 1-acetyl-3-(4-nitrobenzylidene)-2,5-piperazinedione was N-methylated at position 4 by treatment with methyl iodide (2 equivalents) in the presence of  $\text{Na}_2\text{CO}_3$  (1 equivalent) in DMF at room temperature for 30 hours. The resulting N-methylated compound was obtained in 34% yield.

30      (i) Preparation of compounds 127 and 129.

The N-methylated compound was treated with benzaldehyde (1 equivalent) in DMF in the presence of  $\text{Cs}_2\text{CO}_3$  (1 equivalent) at 80°C for 4 hours to give compound 127 in 67% yield. Compound 127 was reduced by 35 hydrogenation at atmospheric pressure over 10% palladium on carbon as catalyst in  $\text{CH}_2\text{Cl}_2$  in the presence of a few drops of trifluoroacetic acid at room temperature for 16 hours to

- 52 -

give compound 129 in 17% yield.

(ii) Preparation of compounds 171, 152 and 137

The N-methylated compound was treated with 4-methoxybenzaldehyde (1 equivalent) in DMF in the presence 5 of Cs<sub>2</sub>CO<sub>3</sub> (1 equivalent) at 80°C for 4 hours to give compound 171 in 38% yield. Compound 171 was reduced by hydrogenation at atmospheric pressure over 10% palladium on carbon as catalyst in CH<sub>2</sub>Cl<sub>2</sub> in the presence of a few drops of trifluoroacetic acid at room temperature for 16 10 hours to give compound 152 in 95% yield. Compound 152 was acetylated by treatment with acetic anhydride in the presence of triethylamine and DMAP to give compound 137 in low yield.

15 Example 9: Preparation of compounds 135 and 136

1-Acetyl-3-(2-nitrobenzylidene)-2,5-piperazinedione was N-methylated at position 4 in 31% yield by treatment with sodium hydride (1.1 equivalents) in THF at 0°C for 60 minutes and then with methyl iodide (5.0 equivalents) at 20 room temperature for 18 hours. Subsequent treatment with 4-methoxybenzaldehyde (1 equivalent) in DMF in the presence of Cs<sub>2</sub>CO<sub>3</sub> at 90°C for 2 hours gave compound 136 in 32% yield.

By an analogous process starting from 1-acetyl-3-(2,6-dichlorobenzylidene)-2,5-piperazinedione compound 135 was obtained. The yields were 26% for the initial N-methylation and 47% for the subsequent condensation with aldehyde.

30 Example 10: Preparation of compounds 166 and 167

1-Acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione was treated with bromomethylcyclopropane (1 equivalent) in DMF in the presence of Na<sub>2</sub>CO<sub>3</sub> (1 equivalent) at 50°C for 48 hours. The product obtained in 11% yield, 35 was condensed with benzaldehyde (1.0 equivalents) in DMF in the presence of Cs<sub>2</sub>CO<sub>3</sub> (1.1 equivalents) at 90°C for 2-5 hours to give compound 167 in 30% yield.

- 53 -

Similarly, compound 166 was prepared in 29% yield by treating 1-acetyl-3-benzylidene-2,5-piperazinedione with bromomethylcyclopropane (1.04 equivalents) in DMF in the presence of Na<sub>2</sub>CO<sub>3</sub> (1.1 equivalents) at 80-85°C for 5 hours. The product was obtained in 12% yield. It was then condensed with 4-methoxybenzaldehyde (1.2 equivalents) in DMF in the presence of Cs<sub>2</sub>CO<sub>3</sub> (1.1 equivalents) at 80°C for 2 hours to give compound 166 in 29% yield.

10 **Example 11: Preparation of compound 148**

(3Z,6Z)-3-(4-acetoxymethylbenzylidene)-6-benzylidene-2,5-piperazinedione was treated with sodium hydride (2.0-3.5 equivalents) in DMF at 0°C for 10-90 minutes followed by methyl iodide (2.0-5.0 equivalents) in DMF at room temperature for 3-48 hours. Removal of solvent in vacuo and flash chromatography of the residue gave compound 148 in 32% yield and the corresponding compound wherein R<sub>11</sub>=R<sub>12</sub>=Me, R<sub>7</sub>=CH<sub>2</sub>OH R<sub>1</sub>-R<sub>6</sub> and R<sub>8</sub>-R<sub>10</sub>=H in 30% yield.

20 **Example 12: Preparation of compounds 140 and 158**

(3Z,6Z)-3-Benzylidene-6-(1-naphthylmethylene)-2,5-piperazinedione, which may be prepared by treating 1-acetyl-3-benzylidene-2,5-piperazinedione with 1-naphthaldehyde (1 equivalent) in DMF in the presence of Cs<sub>2</sub>CO<sub>3</sub> (1.0-1.1 equivalents) at 80-100°C for 1 to 6 hours, was methylated by treatment with NaH (2.0-3.5 equivalents) in DMF at 0°C for 10-90 minutes followed by methyl iodide in DMF at room temperature for 3-48 hours. Removal of solvent in vacuo and flash chromatography of the residue gave compound 140 in 51% yield.

By an analogous method, compound 158 was prepared by methylation of (3Z,6Z)-3-benzylidene-6-(2-N-methyltrimethylacetamidobenzylidene)-2,5-piperazinedione in 49% yield.

35

**Example 13: Interconversions of compounds A**

(i) Compound (1) prepared as described in Example 1 was

- 54 -

treated with  $\text{BBr}_3$  (10.0 equivalents) in  $\text{CH}_2\text{Cl}_2$  at room temperature for 2-5 hours to give compound 123 in 45% yield.

(ii) Compound 122 was treated with sodium hydride, then 5 ethyl iodide (1 equivalent) in DMF at room temperature overnight. The solvent was removed in vacuo and the residue purified by flash chromatography to give compound 169 in 40% yield.

(iii) Compound (1) was treated with 10 bromomethylcyclopropane (1.5 equivalents) in DMF in the presence of  $\text{Na}_2\text{CO}_3$  (1.0 equivalents) at 85°C for 4 hours to give compound 168 in 11% yield.

15 **Example 14 Pharmaceutical composition**

Tablets, each weighing 0.15 g and containing 25 mg of a compound of the invention can be manufactured as follows: Composition for 10,000 tablets  
compound of the invention (250 g)

20 lactose (800 g)  
corn starch (415 g)  
talc powder (30 g)  
magnesium stearate (5 g)

The compound of the invention, lactose and half of 25 the corn starch are mixed. The mixture is then forced through a sieve 0.5 mm mesh size. Corn starch (10 g) is suspended in warm water (90 ml). The resulting paste is used to granulate the powder. The granulate is dried and broken up into small fragments on a sieve of 1.4 mm mesh 30 size. The remaining quantity of starch, talc and magnesium stearate is added, carefully mixed and processed into tablets.

**Example 15: Testing of compounds A as modulators of MDR**

35 **Materials and Methods**

The EMT6 mouse mammary carcinoma cell line and the MDR resistant subline AR 1.0 were cultured in RPMI 1640

- 55 -

medium containing 10% foetal calf serum and 2mM glutamine at 37°C in 5% CO<sub>2</sub>. Cells were passaged between 1 in 200 and 1 in 2000 in the case of the parental cell line and between 1 in 20 and 1 in 200 in the case of the MDR 5 resistant subline, after trypsinisation (0.25% trypsin, 0.2g l<sup>-1</sup>, EDTA).

1. Drug accumulation assay

AR 1.0 cells were seeded into 96 well opaque culture 10 plates (Canberra Packard). The assay medium contained a mixture of tritiated Daunorubicin (DNR), a cytotoxic agent, and unlabelled DNR (0.25 μ Ci/ml; 2μM). Compounds of formula A were serially diluted in assay medium over a range of concentrations from 100 nM to 100 μM. The cells 15 were incubated at 37°C for 1 hr before washing and counting of cell associated radioactivity. Each assay included a titration of the known resistance modifying agent Verapamil as positive control. Results were expressed as % maximum accumulation where 100% accumulation is that observed in 20 the presence of 100μM Verapamil.

The results are set out in the following Table 3.

- 56 -

TABLE 3

Compound No.	Accumulation IC <sub>50</sub> (μM) or % max	Compound No.	Accumulation IC <sub>50</sub> (μM) or % max
1	20 μM		
121	80 μM	122	100 μM
124	32% max	126	45% max
131	50 μM	132	8 μM
133	39% max	134	50% max
135	15 μM	136	36%
137	44% max	138	100 μM
139	30 μM	140	10 μM
140	10 μM	141	25 μM
142	40 μM	143	45% max
144	25 μM	145	35 μM
146	35 μM	147	40 μM
148	65 μM	149	30 μM
150	50 μM	151	20 μM
153	80 μM	154	30 μM
155	20 μM	156	30% max
157	30 μM	158	20 μM
159	15% max	160	35 μM
161	20 μM	162	15 μM
163	20 μM	164	20 μM
165	7 μM	166	10 μM
167	10 μM	168	12 μM
169	25 μM	170	10 μM

2. Potentiation of Doxorubicin toxicity

Compounds of formula A were examined for their ability to potentiate the toxicity of doxorubicin in AR 1.0 cells. In initial proliferation assays compounds were titrated against a fixed concentration of doxorubicin (0.5-1μM) which alone is non-toxic to AR 1.0 cells. Incubations with doxorubicin were over a four day period before

- 57 -

quantitation of proliferation using the colorimetric sulphorhodamine B assay (Skehan *et al*; J. Natl. Cancer Inst. 82 pp 1107-1112 (1990))

Compounds which were shown to be able to sensitise  
 5 AR 1.0 cells to 0.8-1.7  $\mu\text{M}$  doxorubicin without high innate toxicity were selected for further study. Cells were cultured for four days with concentrations of doxorubicin over the range of 0.5 nM-50  $\mu\text{M}$  in the presence of Verapramil at its maximum subtoxic level determined from  
 10 previous experiments. Proliferation was quantified as described by Skehan *et al*, loc cit. The  $\text{IC}_{50}$  (concentration required to reduce proliferation to 50% of the untreated controls) for doxorubicin alone and with the Verapamil were derived and used to calculate the  
 15 potentiation index (PI):

$$\text{PI} = \frac{\text{IC}_{50} \text{ for Doxorubicin alone}}{\text{IC}_{50} \text{ for Doxorubicin plus RMA}}$$

20

TABLE 4

Compound	Potentiation Index
164	7.5 (at 3 $\mu\text{M}$ )
166	10 (at 3 $\mu\text{M}$ )
166	10 (at 5 $\mu\text{M}$ )
167	6 (at 3 $\mu\text{M}$ )
168	20 (at 3 $\mu\text{M}$ )

30 Example 16: Characterization of compounds of formula A

The compounds prepared in Examples 1 to 13 were characterised by conventional mass spectroscopic, microanalytical, proton nmr and i.r. techniques. The results are set out in Table 5.

**TABLE 5**

Nº	Mol. Formula (M. Wt)	Mass spec m/z, intensity (mode)	1Hnmr 6 All 400 MHz	Solvent 6	Microanalysis calc	Infrared Found
121	$C_{20}H_{16}N_2O_3 = 334$	335, $M^+$ , 100% 276 (10%), 259 (20%), 217 (20%), 154 (20%). (DCI/NH <sub>3</sub> )	d <sub>6</sub> -DMSO 10.38 (s, 1H), 7.55 (d, 2H), 7.45-7.32 (m, 5H), (s, 1H), (d, 2H), (s, 1H), (s, 3H), (s, 3H).	C H N	71.84 5.43 8.38	71.10 5.37 8.37
122	$C_{19}H_{16}O_2N_2 = 304$	305, $M^+$ , 100% (DCI/NH <sub>3</sub> )	CDCl <sub>3</sub> , 3.00 (3H, s), 7.07 (1H, s), 7.28 (11H, M), 7.99 (1H, Broad singlet).	C H N	74.98 5.30 9.20	74.54 5.24 9.12
123	$C_{19}H_{16}N_2O_3 = 320$	320, $M^+$ , 100% (EI <sup>+</sup> )	CDCl <sub>3</sub> +CF <sub>3</sub> CO <sub>2</sub> D 3.14 (3H, s) 6.90 (2H, d, $J=8\text{Hz}$ ), (2H, d, $J=8\text{Hz}$ ), 7.35 - 7.50 (7H, m).	C H N	71.24 5.03 8.74	70.62 4.97 8.56

## **SUBSTITUTE SHEET**

124	C <sub>21</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	380, MNH <sub>4</sub> <sup>+</sup> , 7% 363, MH <sup>+</sup> , 363 100%	CDCl <sub>3</sub> DCI/NH <sub>3</sub>	3.00 (3H, s), 3.91 (3H, s), 7.10 (1H, s), 7.29 (1H, s), 7.34 (2H, d, J=6Hz), 7.35 - 7.49 (5H, m), 8.04 (2H, d, J=6Hz).	C H N	69.60 5.01 7.73	68.84 5.03 7.64	68.70 5.01 7.64
125	C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>4</sub>	366, MNH <sub>4</sub> <sup>+</sup> , 1% 349 (MH <sup>+</sup> , 100%), 279 (17%)	d <sub>6</sub> -DMSO DCI/NH <sub>3</sub>	2.86 (3H, s), 6.85 (1H, s), 7.09 (1H, s), 7.32 (1H, m), 7.38-7.50 (4H, m), 7.58 (2H, d, J=7Hz), 7.95 (2H, J=7Hz), 8.53 (1H, br.s).	C H N	68.96 4.63 8.04	68.54 4.65 7.81	68.65 4.71 7.99
126	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub>	366, MNH <sub>4</sub> <sup>+</sup> , 2% 349, MH <sup>+</sup> , 100%	CDCl <sub>3</sub>	3.00 (6H, s) 4.78 (2H, s) 7.25 (2H, s) 7.45 (9H, s)				
127	C <sub>19</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub> = 349	350, MH <sup>+</sup> , 100% 367, MNH <sub>4</sub> <sup>+</sup> , 10% 320 (10%)	CDCl <sub>3</sub> DCI/NH <sub>3</sub>	8.25 (d, 2H), 8.11 (s, 1H), 7.49-7.38 (m, 7H), 7.28 (s, 1H), 7.11 (s, 1H), 3.01 (s, 3H).	C H N	65.32 4.33 12.03	65.03 4.28 11.87	65.31 4.25 11.94

128	$C_{24}H_{23}N_3O_3$	419, $MNH_4^+$ , 1% 402, $MH^+$ , 100% DCI/ $NH_3$	CDCl <sub>3</sub> 1.85-2.05 (4H, m), 3.00 (3H, s), 3.46 (2H, m), 3.68 (2H, m), 7.09 (1H, s), 7.29 (1H, s), 7.30 (2H, d, J=8Hz), 7.32-7.52 (5H, m), 7.58 (2H, d, J=8Hz), 8.01 (1H, br.s).	
129	$C_{19}H_{17}N_3O_2 = 319$	320, $MH^+$ , 100% DCI/ $NH_3$	CDCl <sub>3</sub> 7.93 (s, 1H), 7.48 - 7.42 (m, 5H), 7.21 (s, 1H), 7.12 (d, 2H), 7.05 (s, 1H), 6.68 (d, 2H), 3.08 (s, 3H).	
130	$C_{20}H_{17}N_3O_3 = 319$	365, $MNH_4^+$ , 11% 348, $MH^+$ , 100% DCI/ $NH_3$	d <sub>6</sub> -DMSO 2.85 (3H, s), 6.86 (1H, s), 7.09 (1H, s), 7.24-7.49 (6H, m), 7.52 (2H, d, J=6Hz), 7.87-8.01 (3H, m), 10.50 (1H, br.s).	C 69.15 H 4.93 N 12.10 d <sub>6</sub> -DMSO 68.47 H 4.96 N 11.57 d <sub>6</sub> -DMSO 68.53 H 4.95 N 11.59

131	$C_{22}H_{21}N_3O_4 = 391$	409, 392, $MNH_4^+$ , $MH^+$ , 100% DCI/NH <sub>3</sub>	$CDCl_3+CF_3CO_2H$ 2.32 (3H, s), 3.11 (3H, s), 3.89 (3H, s), 6.96 (2H, d), 7.19 (1H, s), 7.28 (2H, d), 7.40 (1H, s), 7.48 (2H, d), 7.59 (2H, d).	
132	$C_{20}H_{16}N_2O_3Cl_2 = 402$	403/405/407, (100/64/13) %	$CDCl_3$ 3.06 (3H, s), 3.84 (3H, s), 6.90-6.93 (3H, m), 7.23-7.28 (4H, m), 7.40 (2H, d)	C H N Cl 59.57 4.00 6.95 17.58 59.37 4.11 7.01 16.95 59.48 4.11 7.05 16.95
133	$C_{20}H_{17}N_3O_5 = 379$	380, 397, 100% 76%	$CDCl_3$ 3.06 (3H, s), 3.87 (3H, s), 6.44 (2H, d), 7.18 (1H, s), 7.23-7.28 (2H, m), 7.32 (1H, s), 7.51-7.59 (2H, m), 7.73 (1H, t), 8.01 (1H, bs), 8.20 (1H, d)	

- 62 -

134	$C_{23}H_{23}N_3O_4 = 405$	423, 406, MH <sup>+</sup> , DCI/NH <sub>3</sub>	MNH <sub>4</sub> <sup>+</sup> , MH <sup>+</sup> , 100% 60% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> +CF <sub>3</sub> CO <sub>2</sub> H 2.32(3H,s), 3.06(3H,s), 3.3(3H,s), 3.86(3H,s), 6.92(2H,d), 7.02(1H,s), 7.22-7.39 (6H,m)', 7.49(2H,d)	C H N	59.57 4.00 6.95	59.03 3.95 6.94	59.01 3.88 7.01
135	$C_{20}H_{16}N_2O_3Cl_2 = 402$	403, (93, 367, 373, (29, (39) DCI/NH <sub>3</sub>	405, 100, 46)% 369, 371, 59, 51, 59, 7.21-7.26 (1H, m)', 7.36(4H, m)', 8.06(1H, very broad s)	CDCl <sub>3</sub> 2.89(3H,s), 3.87(3H,s), 6.98(2H,d), 7.04(1H,s), 7.07(1H,s), 7.21-7.26(1H, m)', 7.36(4H, m)', 8.06(1H, very broad s)	C H N	59.57 4.00 6.95	59.03 3.95 6.94	59.01 3.88 7.01
136	$C_{20}H_{17}O_5N_3 = 379$	397, 380, 333, DCI/NH <sub>3</sub>	16% 100% 49% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 2.90(3H,s), 3.86(3H,s), 6.99(2H,d), 7.06(1H,s), 7.30-7.40(3H, m)', 7.45(1H,s), 7.53(1H,t), 7.66(1H,t), 8.07(1H, very broad s)', 8.19(1H,d)	C H N	63.32 4.52 11.08	63.06 4.46 10.70	62.95 4.47 10.76

137	$C_{22}H_{21}N_3O_4 = 391$	392, 409, $MH^+$ , $MNH_2^+$ , $MNH_3^+$ , 10% $DCI/NH_3$	$d_6$ -DMSO 10.28 (s,1H), 10.00 (s,1H), 7.61 (d,2H), 7.55 (d,2H), 7.28 (d,2H), 7.00 - 7.03 (m,3H), (s,1H), (s,3H), (s,3H), (s,3H), 100% $MH^+-HCl$ ,	
138	$C_{25}H_{30}N_5O_4Cl$	472, 436, 100% $MH^+$ , $MNH_2^+$ , $MNH_3^+$ , 10% $CDCl_3$	2.46 (2H,m), 2.88 (6H,d, $J=3Hz$ ), 3.05 (3H,s), 3.27 (2H,m), 3.88 (3H,s), 4.16 (2H,m), 6.90- 7.01 (5H,m), 7.20-7.28 (2H,m), (2H,d, $J=7Hz$ ), 7.90 (1H,br.s), 12.90 (1H,br.s). (under $CDCl_3$ peak)	

No	Mol. Formula (M. Wt)	Mass spec m/z, intensity (mode)	<sup>1</sup> Hnmr δ All 400 MHz	Microanalysis			Infrared
				Solvent	calc	Found	
139	C <sub>21</sub> H <sub>17</sub> N <sub>2</sub> O <sub>2</sub> F <sub>3</sub> = 386	387, MH <sup>+</sup> , 100% 319 (<10%) DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 7.65 (d, 2H), 7.47 (d, 2H), 7.45-7.33 (m, 6H), (s, 1H), (s, 3H), (s, 3H).	C H N	65.28 4.43 7.25	65.46 4.56 7.10	65.44 4.59 7.12
140	C <sub>24</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> = 368	368, M <sup>+</sup> , 20% 369, MH <sup>+</sup> , 100% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 8.05 (d, 1H), 7.91-7.84 (m, 2H), (s, 1H), 7.32 (m, 9H), 7.30 (s, 1H), 3.03 (s, 3H), 2.77 (s, 3H).				
141	C <sub>22</sub> H <sub>23</sub> N <sub>3</sub> O <sub>2</sub> = 361	362, MH <sup>+</sup> , 100% 257, 55% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 7.37-7.30 (m, 5H), (s, 1H), (s, 1H), (d, 2H), (d, 2H), (s, 3H), (s, 6H), (s, 3H).				

142	$C_{21}H_{20}N_2O_3 = 348$	349, $MH^+$ , 100% 366, $MNH_4^+$ , 5% 317(15%) DCI/ $NH_3$	CDCl <sub>3</sub> 7.41-7.31 (m, 6H), 7.30 (s, 1H), 7.23 (s, 1H), 7.20 (m, 1H), 7.0 - 6.9 (m, 2H), 3.85 (s, 3H), 2.99 (s, 3H), 2.92 (s, 3H).
143	$C_{20}H_{19}N_3O_2 = 333$	334, $MH^+$ , 100% 318 (20%), 290 (30%), 277 (20%). DCI/ $NH_3$	CDCl <sub>3</sub> 7.39-7.30 (m, SH), 7.19 (s, 1H), 7.17 (d, 2H), 7.14 (s, 1H), 6.68 (d, 2H), 3.05 (s, 3H), 2.95 (3H), 2.90 (s, b, 2H).
144	$C_{12}H_{17}N_2O_2F = 336$	337 100% DCI/ $NH_3$	CDCl <sub>3</sub> 2.98 (3H, s), 3.00 (3H, s), 7.10-7.44 (11H, m)
145	$C_{20}H_{17}N_2O_2F = 336$	337 100% DCI/ $NH_3$	CDCl <sub>3</sub> 2.98 (6H, overlapping singlets), 7.10 (2H, t), 7.18 (1H, s), 7.25 (1H, s), 7.30-7.42 (9H, m)

146	C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub> F <sub>2</sub>	CDCl <sub>3</sub> 2.96 (3H, s), 2.99 (3H, s), 6.85-6.97 (2H, m), 7.11 (1H, s), 7.24 (1H, s), 7.30-7.41 (6H, m)	C H N 67.79 4.55 7.91 67.66 4.56 7.84 67.67 4.56 7.83
147	C <sub>23</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub>	391, M <sup>+</sup> H, 8% 363 (100%) DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 7.41-7.28 (8H,m), 7.23 (2H,d), 4.48 (2H,s), 3.42 (3H,s), 2.98 (6H,s).
148	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> = 348	349, MH <sup>+</sup> 366, MNH <sub>4</sub> <sup>+</sup> , 2% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 7.45 (9H,m), 7.25 (2H,s), 4.78 (2H,s), 3.00 (6H,s).
149	C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub> = 362	363, MH <sup>+</sup> , 100% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 7.41-7.28 (m,7H), 7.20 (s,1H), 7.18 (s,1H), 6.92 (d,2H), 4.08 (q,2H), 3.02 (s,3H), 2.96 (s,3H), 1.42 (t,3H).

150	$C_{20}H_{18}N_2O_2 = 318$	319, $MH^+$ , 100% DCI/ $NH_3$	CDCl <sub>3</sub> 7.41-7.31 (m, 10H), 7.24 (s, 2H), 2.99 (s, 6H).			
151	$C_{20}H_{16}N_2O_2Cl_2 = 387$	387, $MH^+$ , 100% 389 (60%), 391 (10%), MNH <sub>4</sub> <sup>+</sup> 404 (<10%), 351, 353, 355; 338; 324; 310 DCI/ $NH_3$	CDCl <sub>3</sub> 7.41-7.30 (m, 7H), 7.28 (s, 1H), 7.26 (m, 1H), 7.05 (s, 1H), 2.99 (s, 3H), 2.78 (s, 3H).	C 62.03 H 4.16 N 7.23	61.89 4.11 7.09	61.95 4.10 7.10
152	$C_{20}H_{19}N_3O_3$	350, $MH^+$ , 100%	CDCl <sub>3</sub> 3.08 (s, 3H) 3.83 (s, 3H) 6.68 (d, 2H) 6.95-7.00 7.13 (d, 2H) 7.20 (s, 1H) 7.38 (d, 2H) 7.85 (s, 1H)			
153	$C_{23}H_{23}N_3O_3 = 389$	390, $MH^+$ , 100% 407, MNH <sub>4</sub> <sup>+</sup> , 10% 389 (60%), 391 (20%), 347 (10%) DCI/ $NH_3$	CDCl <sub>3</sub> 7.40-7.30 (m, 7H), 7.26-7.20 (m, 4H), 3.29 (s, 3H), 2.99 (s, 3H), 1.95 (s, broad, 3H).	C 70.93 H 5.95 N 10.79	70.82 5.97 10.76	70.71 5.95 10.74

154	$C_{20}H_{16}N_2O_2Cl_2$	404, 406, 408, $(M+NH_4)^+$ , 2%, 387, 389, 391, $(M+H)^+$ , 100%	CDCl <sub>3</sub> , 7.45 (1H, d), 7.42-7.28 (6H, m), 7.15 (2H, dd), 7.09 (1H, s), 2.99 (3H, s), 2.97 (3H, s).		
155	$C_{20}H_{17}N_2O_2Cl =$ 352.5	353, MH <sup>+</sup> , 100% 355 (40%), 319 (10%) DCI/NH <sub>3</sub>	CDCl <sub>3</sub> , 7.41-7.30 (m, 8H), 7.27 (s, 1H), 7.22 (s, 1H), 7.17 (s1H), 2.98 (s, 6H)	C H N	68.09 4.86 7.94 67.83 4.96 7.60 67.86 5.00 7.67
156	$C_{21}H_{20}N_2O_3S = 380$	381, MH <sup>+</sup> , 100% (50%) 365 DCI/NH <sub>3</sub>	CDCl <sub>3</sub> , 7.68 (d, 2H), 7.50 (d, 2H), 7.44-7.31 (m, 6H), 7.22 (s, 1H), 2.99 (s, 3H), 2.98 (s, 3H), 2.77 (s, 3H).		

**SUBSTITUTE SHEET**

- 69 -

157	$C_{22}H_{23}N_3O_4S = 425$	426, MH+, 100% 443, $MNH_4^+$ , 10% (15%), 412 DCI/ $NH_3$	CDCl <sub>3</sub> 7.81 (d, 2H), 7.49 (d, 2H), 7.41-7.31 (m, 5H), 7.28 (s, 1H), 7.22 (s, 1H), 2.99 (s, 3H), 2.97 (s, 3H), 2.74 (s, 6H)	C H N	62.09 5.45 9.88	61.20 5.54 9.34	61.14 5.50 9.28
158	$C_{26}H_{29}N_3O_3 = 431$	432, MH+, 100% 330 (10%), 346 (10%)	CDCl <sub>3</sub> 7.41-7.30 (m, 8H), 7.23 (s, 1H), 7.22 (m, 1H), 7.11 (s, 1H), 3.28 (s, 3H), 3.02 (s, 3H), 2.97 (s, 3H), 1.2 (s, 9H).				
159	$C_{26}H_{22}N_2O_2$	395, MH+, 100% DCI/ $NH_3$	CDCl <sub>3</sub> 7.67-7.58 (4H, m), 7.48- 7.29 (9H, m), 7.26-7.21 (3H, m), 3.06 (3Hs), 2.98 (3H, s).				

160	$C_{22}H_{20}N_2O_4$	377, 100% DCI/NH <sub>3</sub>	(M+H) <sup>+</sup> , 8.05 (2H, d), 7.42-7.29 (8H, m), 7.22 (1H, s), 2.99 (3H, s), 2.96 (3H, s).	CDCl <sub>3</sub>	
161	$C_{20}H_{17}N_2O_2Br =$ $397 \pm 1$	397:399, 100% DCI/NH <sub>3</sub>	d <sub>6</sub> -DMSO 2.86 (6H, two singlets of virtually identical shift), 7.01 (1H, s), 7.08 (1H, s), 7.31-7.45 (7H, m), 7.60 (2H, d)		
162	$C_{21}H_{18}N_2O_3F_2 =$ $384$	385 100%	CDCl <sub>3</sub> 2.97 (3H, s), 3.02 (3H, s), 3.86 (3H, s), 6.85-6.96 (4H, m), 7.09 (1H, s), 7.19 (1H, s), 7.23-7.30 (3H, m)		

- 71 -

163	$C_{27}H_{19}N_2O_3Br = 427 \pm 1$	427:429 100:100% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 2.97 (3H, s), 3.02 (3H, s), 3.85 (3H, s), 6.92 (2H, d), 7.12 (1H, s), 7.17 (3H, m), 7.23-7.30 (2H, m) 7.53 (2H, d)	C H N	59.03 4.48 6.56	58.85 4.46 6.46	58.79 4.49 6.47
164	$C_{21}H_{19}N_2O_3F = 366$	367 100% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 2.98 (3H, s), 3.01 (3H, s), 3.85 (3H, s), 6.93 (2H, d), 7.10 (2H, m), 7.18 (2H, d), 7.26-7.35 (4H, m)	C H N	68.84 5.23 7.65	68.42 5.19 7.55	68.47 5.27 7.54
165	$C_{20}H_{15}N_3O_4Cl_2 = 431$	449/451/453 (9:6:1) 100% 432/434/436 (9:6:1) 64% 402 24%	CDCl <sub>3</sub> 2.81 (3H, s), 2.85 (3H, s), 7.09 (1H, s), 7.23-7.29 (1H, m), 7.33-7.40 (3H, m), 7.47 (1H, s), 7.56 (1H, t), 7.69 (1H, t), 8.17 (1H, d)	C H N	55.57 3.50 9.72	55.34 3.48 9.56	55.36 3.50 9.55

## **SUBSTITUTE SHEET**

Nº	Mol. Formula (M. Wt)	Mass spec <i>m/z</i> , intensity (mode)	<sup>1</sup> Hnmr		Microanalysis		Infrared	
			Solvent <i>δ</i>	All 400 MHz	calc	Found		
166	C <sub>23</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub> = 374	375, M <sup>+</sup> H, 100% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 0.02-0.08 (2H, m), 0.47 (2H, m), 0.95-1.04 (1H, m), (2H, d), (3H, s), (2H, d), (1H, s), (1H, s), (1H, s), (1H, s), (1H, s), (1H, broad, s).	0.43- 0.43- 0.43- 0.43- 3.55 3.85 6.98 7.02 7.22 7.30- 7.40 (7H, m), 7.46 7.48 7.59 5.77 5.90 7.44	C H N	73.78 5.92 7.48	73.59 5.77 7.46	73.56 5.90 7.44

167	C <sub>23</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub>	375, MH <sup>+</sup> , 100% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 0.10 (2H, m), 0.39 (2H, m), 1.05 (1H, m), 3.58 (2H, d, J=6Hz), 3.86 (3H, s), 6.90 (2H, d, J=7Hz), 7.05 (1H, s), 7.20 (1H, s), 7.28 (2H, d, J=7Hz), 7.35 (1H, m), 7.43 (4H, m), 7.87 (1H, br.s)	
168	C <sub>24</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>	389, 100% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 0.09 (2H, m), 0.37 (2H, m), 1.00 (1H, m), 3.05 (3H, s), 3.49 (2H, d), 3.35 (3H, s), 6.42 (2H, d), 7.16 (2H, d), 7.27-7.40 (7H, m)	
169	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub>	333, MH <sup>+</sup> , 100% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> 7.42-7.29 (10H, m), 7.19 (2H, d), 3.62 (2H, q), 2.98 (3H, s), 0.99 (3H, t).	

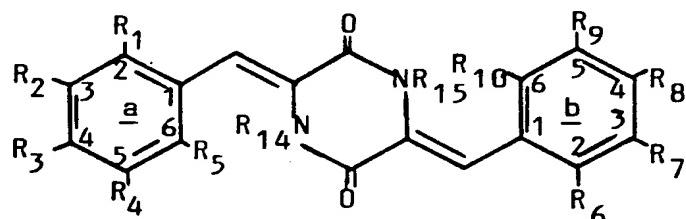
170	C <sub>24</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>	389, MH <sup>+</sup> , 100% 333, 305, A11 <10% DCI/NH <sub>3</sub>	7.40-7.30 (m, 7H), 7.19 (s, 1H), 7.11 (s, 1H), 6.92 (d, 2H), 3.85 (s, 3H), 3.52 (d, 2H), 3.00 (s, 3H), 1.01 (m, 1H), 0.48 (m, 2H), 0.09 (m, 2H).	C H N	74.21 6.23 7.21	73.95 6.24 7.15	74.20 6.28 7.26	
171	C <sub>20</sub> H <sub>17</sub> N <sub>3</sub> O <sub>5</sub>	397, MNH <sub>4</sub> <sup>+</sup> , 10% 380, MH <sup>+</sup> , 100% DCI/NH <sub>3</sub>	CDCl <sub>3</sub> +CF <sub>3</sub> CO <sub>2</sub> D					

- 75 -

CLAIMS

1. A diketopiperazine of formula (A):

5



(A)

- 10 wherein each of  $R_{14}$  and  $R_{15}$ , which may be the same or different, is independently selected from hydrogen and  $C_1-C_6$  alkyl provided at least one of  $R_{14}$  and  $R_{15}$  is  $C_1-C_6$  alkyl; and each of  $R_1$  to  $R_{10}$ , which may be the same or different, is independently selected from hydrogen,  $C_1-C_6$  alkyl  
 15 unsubstituted or substituted by one or more halogen atoms,  $C_1-C_6$  alkoxy,  $C_1-C_6$  alkylthio, halogen, hydroxy, nitro, optionally substituted phenyl, cyano,  $-CH_2OH$ ,  $-CH_2COOH$ ,  $-CO_2R^{11}$ ,  $-NHCOR^{11}$ ,  $-NHSO_2R^{13}$ ,  $-SO_2R^{13}$ ,  $-CON(R^{11}R^{12})$ ,  $-SOR^{13}$ ,  
 20  $-SO_2N(R^{11}R^{12})$ ,  $-N(R^{11}R^{12})$ ,  $-O(CH_2)_nN(R^{11}R^{12})$ ,  $-O(CH_2)_nCO_2R^{11}$ ,  $-OCOR^{11}$ ,  $-CH_2OCOR^{11}$ ,  $-CH_2NHCOR^{11}$ ,  $-CH_2NHCOOR^{13}$ ,  $-CH_2SR^{11}$ ,  $-CH_2SCOR^{11}$ ,  $-CH_2S(O)_mR^{13}$  wherein  $m$  is 1 or 2,  
 25  $-CH_2NHCO(CH_2)_nCO_2R^{11}$ ,  $-N(R^{11})COR^{12}$ ,  $-NHCOCF_3$ ,  $-NHCO(CH_2)_nCO_2R^{11}$ ,  $-NHCO(CH_2)_nOCOR^{11}$  and  $-NHCO(CH_2)_nOR^{11}$  wherein  $n$  is 0 or is an integer of from 1 to 6, each of  $R^{11}$  and  $R^{12}$  is independently  
 30  $H$  or  $C_1-C_6$  alkyl or, when  $R^{11}$  and  $R^{12}$  are attached to the same nitrogen atom, they may alternatively form with the nitrogen atom a saturated five or six-membered heterocyclic ring; and  $R^{13}$  is  $C_1-C_6$  alkyl; or any of  $R_1$  and  $R_2$ ,  $R_2$  and  $R_3$ ,  $R_3$  and  $R_4$  and  $R_4$  and  $R_5$ , or  $R_6$  and  $R_7$ ,  $R_7$  and  $R_8$ ,  $R_8$  and  $R_9$  and  $R_9$  and  $R_{10}$ , form together with the carbon atoms to which they are attached a benzene ring which is optionally substituted; or a pharmaceutically acceptable salt or ester thereof; with the exception of compounds wherein:  
 (i) each of  $R_1$  to  $R_{10}$  is  $H$ ; and  
 35 (ii)  $R_{14}$  and  $R_{15}$  are both Me,  $R_8$  is OMe and the rest of  $R_1$  to  $R_{10}$  are  $H$ .

2. A compound according to claim 1 wherein one of

- 76 -

$R_{14}$  and  $R_{15}$  is Me, Et or cyclopropylmethyl and the other is hydrogen, Me, Et or cyclopropylmethyl.

3. A compound according to claim 1 or 2 wherein one of  $R_6$  to  $R_{10}$  is selected from hydroxy, halogen, alkoxy, 5  $-NHCOR^{11}$ ,  $-CO_2R^{11}$ ,  $-SO_2R^{13}$ ,  $-CON(R^{11}R^{12})$ ,  $-NO_2$ ,  $-SO_2N(R^{11}R^{12})$ ,  $-SOR^{13}$ , and  $-N(R^{11})COR^{12}$  and the other four of  $R_6$  to  $R_{10}$  are H.

4. A compound according to claim 3 wherein  $R^8$  is selected from hydroxy, halogen, alkoxy,  $-NHCOR^{11}$ ,  $-CO_2R^{11}$ , 10  $-SO_2R^{13}$ ,  $-CON(R^{11}R^{12})$ ,  $-NO_2$ ,  $-SO_2N(R^{11}R^{12})$ ,  $-SOR^{13}$ , and  $-N(R^{11})COR^{12}$  and  $R_6$ ,  $R_7$ ,  $R_9$  and  $R_{10}$  are H.

5. A compound according to any one of the preceding claims wherein  $R_1$  is H, halogen or  $NO_2$ ;  $R_2$  is H;  $R_3$  is H,  $-NHCOR^{11}$ ,  $N(R^{11})COR^{12}$ ,  $C_1-C_6$  alkoxy or halogen;  $R_4$  is H and  $R_5$  is H or halogen.

15 6. A compound according to any one of claims 1 to 3 wherein any two adjacent groups of  $R_1$  to  $R_{10}$  form, together with the carbon atoms to which they are attached, an optionally substituted benzene ring.

7. A compound according to claim 1 selected from: 20  $(3Z,6Z)$ -3-benzylidene-6-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione;

$(3Z,6Z)$ -6-benzylidene-3-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione;

$(3Z,6Z)$ -3,6-dibenzylidene-1-methyl-2,5-piperazinedione;

25  $(3Z,6Z)$ -3-benzylidene-6-(4-hydroxybenzylidene)-1-methyl-2,5-piperazinedione;

4-(( $3Z,6Z$ )-3-benzylidene-1-methyl-2,5-dioxopiperazin-6-ylidene)methylbenzoic acid, methyl ester;

4-(( $3Z,6Z$ )-3-benzylidene-1-methyl-2,5-dioxopiperazin-6-30 ylidene)methylbenzoic acid;

$(3Z,6Z)$ -3-(3-hydroxymethylbenzylidene)-6-benzylidene-1,4-dimethyl-2,5-piperazinedione;

$(3Z,6Z)$ -3-benzylidene-1-methyl-6-(4-nitrobenzylidene)-2,5-piperazinedione;

35  $N,N$ -tetramethylene-4-(( $3Z,6Z$ )-3-benzylidene-1-methyl-2,5-dioxopiperazin-6-ylidene)methylbenzamide;

$(3Z,6Z)$ -6-(4-aminobenzylidene)-3-benzylidene-1-methyl-2,5-

- 77 -

- piperazinedione;
- 4-((3Z,6Z)-3-Benzylidene-1-methyl-2,5-dioxopiperazin-6-ylidene)methylbenzamide;
- (3Z,6Z)-3-(4-Acetamidobenzylidene)-6-(4-
- 5 methoxybenzylidene)-1-methyl-2,5-piperazinedione;
- (3Z,6Z)-3-(2,6-Dichlorobenzylidene)-6-(4-
- methoxybenzylidene)-1-methyl-2,5-piperazinedione;
- (3Z,6Z)-6-(4-Methoxybenzylidene)-1-methyl-3-(2-
- nitrobenzylidene)-2,5-piperazinedione;
- 10 (3Z,6Z)-6-(4-Methoxybenzylidene)-1-methyl-3-(4-N-
- methylacetamidobenzylidene)-2,5-piperazinedione;
- (3Z,6Z)-6-(2,6-Dichlorobenzylidene)-3-(4-
- methoxybenzylidene)-1-methyl-2,5-piperazinedione;
- (3Z,6Z)-3-(4-Methoxybenzylidene)-1-methyl-6-(2-
- 15 nitrobenzylidene)-2,5-piperazinedione;
- (3Z,6Z)-6-(4-Acetamidobenzylidene)-3-(4-
- methoxybenzylidene)-1-methyl-2,5-piperazinedione;
- (3Z,6Z)-3-(4-(3-N,N-Dimethylaminopropoxy)benzylidene)-6-(4-
- methoxybenzylidene)-1-methyl-2,5-piperazinedione,
- 20 hydrochloride;
- (3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(4-
- trifluoromethylbenzylidene)-2,5-piperazinedione;
- (3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(1-naphthylmethylen)-
- 2,5-piperazinedione;
- 25 (3Z,6Z)-6-Benzylidene-3-(4-dimethylaminobenzylidene)-1,4-
- dimethyl-2,5-piperazinedione;
- (3Z,6Z)-6-Benzylidene-3-(2-methoxybenzylidene)-1,4-
- dimethyl-2,5-piperazinedione;
- (3Z,6Z)-3-(4-Aminobenzylidene)-6-benzylidene-1,4-dimethyl-
- 30 2,5-piperazinedione;
- (3Z,6Z)-6-Benzylidene-3-(2-fluorobenzylidene)-1,4-dimethyl-
- 2,5-piperazinedione;
- (3Z,6Z)-6-Benzylidene-3-(4-fluorobenzylidene)-1,4-dimethyl-
- 2,5-piperazinedione;
- 35 (3Z,6Z)-6-Benzylidene-3-(2,4-difluorobenzylidene)-1,4-
- dimethyl-2,5-piperazinedione;
- (3Z,6Z)-3-(4-Acetoxyethylbenzylidene)-6-benzylidene-1,4-

- dimethyl-2,5-piperazinedione;  
(3Z,6Z)-3-(3-Acetoxyethylbenzylidene)-6-benzylidene-1,4-dimethyl-2,5-piperazinedione;  
(3Z,6Z)-6-Benzylidene-3-(4-ethoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione;  
5 (3Z,6Z)-3,6-Dibenzylidene-1,4-dimethyl-2,5-piperazinedione;  
(3Z,6Z)-6-Benzylidene-3-(2,6-dichlorobenzylidene)-1,4-dimethyl-2,5-piperazinedione;  
10 (3Z,6Z)-6-(4-aminobenzylidene)-3-(4-methoxybenzylidene)-1-methyl-2,5-piperazinedione;  
(3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(4-N-methylacetamido benzylidene)-2,5-piperazinedione;  
(3Z,6Z)-6-Benzylidene-3-(3,4-dichlorobenzylidene)-1,4-dimethyl-2,5-piperazinedione;  
15 (3Z,6Z)-6-Benzylidene-3-(3-chlorobenzylidene)-1,4-dimethyl-2,5-piperazinedione;  
(3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(4-methylsulfinylbenzylidene)-2,5-piperazinedione;  
N,N-Dimethyl-4-((3Z,6Z)-6-Benzylidene-1,4-dimethyl-2,5-dioxopiperazin-3-ylidene)methylbenzenesulfonamide;  
20 (3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(2-N-methyltrimethylacetamido benzylidene)-2,5-piperazinedione;  
(3Z,6Z)-6-Benzylidene-1,4-dimethyl-3-(4-phenylbenzylidene)-2,5-piperazinedione;  
25 4-((3Z,6Z)-6-Benzylidene-1,4-dimethyl-2,5-dioxopiperazin-3-ylidene)methylbenzoic acid, methyl ester;  
(3Z,6Z)-6-Benzylidene-3-(4-bromobenzylidene)-1,4-dimethyl-2,5-piperazinedione;  
30 (3Z,6Z)-3-(2,4-Difluorobenzylidene)-6-(4-methoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione;  
(3Z,6Z)-3-(4-Bromobenzylidene)-6-(4-methoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione;  
(3Z,6Z)-3-(4-Fluorobenzylidene)-6-(4-methoxybenzylidene)-1,4-dimethyl-2,5-piperazinedione;  
35 (3Z,6Z)-3-(2,6-Dichlorobenzylidene)-1,4-dimethyl-6-(2-nitrobenzylidene)-2,5-piperazinedione;  
(3Z,6Z)-6-Benzylidene-1-cyclopropylmethyl-3-(4-

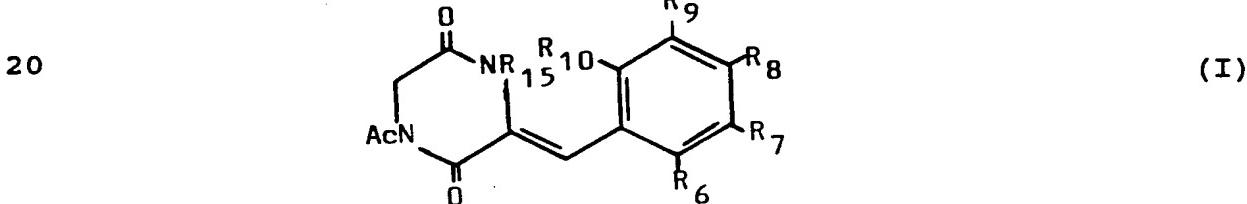
- 79 -

methoxybenzylidene)-2,5-piperazinedione;  
 (3Z,6Z)-3-Benzylidene-1-cyclopropylmethyl-6-(4-methoxybenzylidene)-2,5-piperazinedione;  
 (3Z,6Z)-6-Benzylidene-1-cyclopropylmethyl-3-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione;  
 5 (3Z,6Z)-3,6-Dibenzylidene-1-ethyl-4-methyl-2,5-piperazinedione;  
 (3Z,6Z)-3-Benzylidene-1-cyclopropylmethyl-6-(4-methoxybenzylidene)-4-methyl-2,5-piperazinedione

10 8. A pharmaceutical or veterinary composition comprising a pharmaceutically or veterinarily acceptable carrier or diluent and, as an active principle, a compound as claimed in any one of the preceding claims.

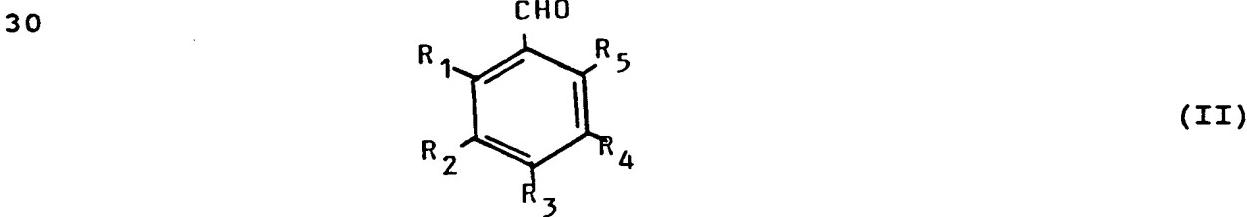
15 9. A process for preparing a compound as defined in claim 1, the process comprising:

(a) condensing a compound of formula (I):



25

wherein R<sub>6</sub> to R<sub>10</sub> and R<sub>15</sub> are as defined in claim 1 and are optionally protected, with a compound of formula (II):



35

wherein R<sub>1</sub> to R<sub>5</sub> are as defined in claim 1 and are

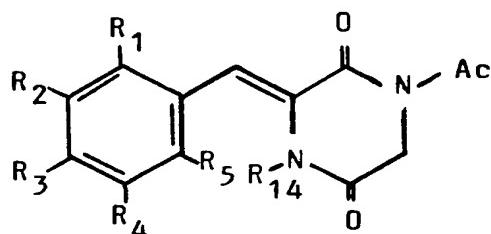
- 80 -

optionally protected, in the presence of a base in an organic solvent thereby obtaining a compound of formula A in which R<sub>14</sub> is hydrogen; or

(b) condensing a compound of formula (I'):

5

10

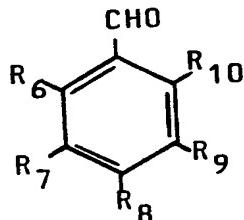


(I')

15

wherein R<sub>1</sub> to R<sub>5</sub> and R<sub>14</sub> are as defined in claim 1 and are optionally protected with a compound of formula (III):

20



(III)

25 wherein R<sub>6</sub> to R<sub>10</sub> are as defined in claim 1 and are optionally protected, in the presence of a base in an organic solvent; and

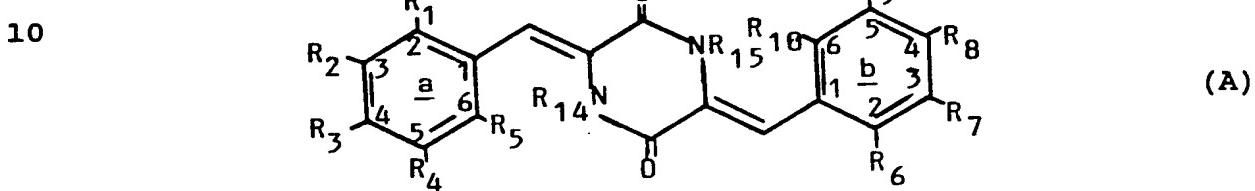
(c) if desired, converting the resulting compound of formula A in which R<sub>14</sub> or R<sub>15</sub>, respectively, is hydrogen  
30 into a corresponding compound of formula A in which R<sub>14</sub> or R<sub>15</sub>, respectively, is a C<sub>1</sub>-C<sub>6</sub> alkyl group, by treatment with an alkylating agent; and/or, if required, removing optionally present protecting groups, and/or, if desired, converting one compound of formula A into another compound  
35 of formula A, into another compound of formula A, and/or, if desired, converting a compound of formula A into a pharmaceutically acceptable salt or ester thereof, and/or,

- 81 -

if desired, converting a salt or ester into a free compound, and/or, if desired, separating a mixture of isomers into the single isomers.

10. A compound as defined in any one of claims 1  
5 to 8 for use as a modulator of multiple drug resistance.

11. Use of a diketopiperazine of formula (A):



15

wherein each of  $R_{14}$  and  $R_{15}$ , which may be the same or different, is independently selected from hydrogen and  $C_1-C_6$  alkyl provided at least one of  $R_{14}$  and  $R_{15}$  is  $C_1-C_6$  alkyl; each of  $R_1$  to  $R_{10}$ , which may be the same or different, is independently selected from hydrogen,  $C_1-C_6$  alkyl unsubstituted or substituted by one or more halogen atoms,  $C_1-C_6$  alkoxy,  $C_1-C_6$  alkylthio, halogen, hydroxy, nitro, optionally substituted phenyl, cyano,  $-CH_2OH$ ,  $-CH_2COOH$ ,  $-CO_2R^{11}$ ,  $-NHCOR^{11}$ ,  $-NHSO_2R^{13}$ ,  $-SO_2R^{13}$ ,  $-CON(R^{11}R^{12})$ ,  $-SOR^{13}$ ,  $-SO_2N(R^{11}R^{12})$ ,  $-N(R^{11}R^{12})$ ,  $-O(CH_2)_nN(R^{11}R^{12})$ ,  $-O(CH_2)_nCO_2R^{11}$ ,  $-OCOR^{11}$ ,  $-CH_2OCOR^{11}$ ,  $-CH_2NHCOR^{11}$ ,  $-CH_2NHCOOR^{13}$ ,  $-CH_2SR^{11}$ ,  $-CH_2SCOR^{11}$ ,  $-CH_2S(O)_mR^{13}$  wherein  $m$  is 1 or 2,  $-CH_2NHCO(CH_2)_nCO_2R^{11}$ ,  $-N(R^{11})COR^{12}$ ,  $-NHCOCF_3$ ,  $-NHCO(CH_2)_nCO_2R^{11}$ ,  $-NHCO(CH_2)_nOCOR^{11}$  and  $-NHCO(CH_2)_nOR^{11}$  wherein 20  $n$  is 0 or an integer of from 1 to 6, each of  $R^{11}$  and  $R^{12}$  is independently H or  $C_1-C_6$  alkyl or, when  $R^{11}$  and  $R^{12}$  are attached to the same nitrogen atom, they may alternatively form with the nitrogen atom a saturated five or six membered heterocyclic ring; and  $R^{13}$  is  $C_1-C_6$  alkyl; or any 25 of  $R_1$  and  $R_2$ ,  $R_2$  and  $R_3$ ,  $R_3$  and  $R_4$  and  $R_4$  and  $R_5$ , or  $R_6$  and  $R_7$ ,  $R_7$  and  $R_8$ ,  $R_8$  and  $R_9$  and  $R_9$  and  $R_{10}$ , form together with the 30 carbon atoms to which they are attached a benzene ring 35

- 82 -

which is optionally substituted; or a pharmaceutically acceptable salt or ester thereof; in the manufacture of a medicament for use as a modulator of multiple drug resistance.

5        12.      Use according to claim 11, wherein the compound is a compound as defined in any of claims 1 to 7.

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 93/01735

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 C07D241/08 A61K31/495

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 5 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN vol. 59 , 1986 , TOKYO JP pages 3917 - 3923 CHUNG-GI SHIN ET AL. 'CONVENIENT SYNTHESIS OF 3-AMINOCOUMARIN DERIVATIVES BY THE CONDENSATION OF 1,4-DIACETYL-OR 3-SUBSTITUENT-2,5-PIPERAZINDIONES WITH VARIOUS SALICYLALDEHYDE DERIVATIVES.' see page 3919 - page 3921; example 8C --- -/-	1,2

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*'A' document defining the general state of the art which is not considered to be of particular relevance
- \*'E' earlier document but published on or after the international filing date
- \*'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*'O' document referring to an oral disclosure, use, exhibition or other means
- \*'P' document published prior to the international filing date but later than the priority date claimed

- \*'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*'&' document member of the same patent family

1

Date of the actual completion of the international search

19 November 1993

Date of mailing of the international search report

30.11.93

## Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+ 31-70) 340-3016

## Authorized officer

FRANCOIS, J

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 93/01735

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 69, no. 28, 1968, Columbus, Ohio, US; abstract no. 96654q, R.F.C. BROWN 'SYNTHETIC APPROACHES OF MYCELIANAMIDE.' page 9051 ; see abstract & AUST. J. CHEM. vol. 21, no. 6 , 1968 , CANBERRA pages 1581 - 1599 -----	1
X		1